Vår ref:2016_H_RX_003
Deres ref:2016/9453

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 02.12.16

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på høringen av fornyelsessøknad **EFSA/GMO/RX/003**, for genmodifisert maislinje DAS-59122-7 som gjelder mat, fôr, import og prosessering fra Pioneer Overseas Corporation og Dow Agrosciences Europe Ltd.

Vennligst ta kontakt hvis det er noen spørsmål.

Med vennlig hilsen,

Idun Merete Grønsberg
Forsker II
GenØk-Senter for Biosikkerhet
idun.gronsberg@genok.no

Bidragsytere:

Lise Nordgård
Forsker II
GenØk-Senter for Biosikkerhet

Anne I. Myhr
Direktør
GenØk-Senter for Biosikkerhet

Lilian van Hove
Forsker III
GenØk- Senter for Biosikkerhet

Vår ref:2016_H_RX_003
Deres ref:2016/9453

Vurdering av fornyelsessøknad EFSA/GMO/RX/003 under EU-forordning 1829/2003/EC som gjelder mat, fôr, import og prosessering av genmodifisert mais DAS-59122-7.

Sendt til

Miljødirektoratet

av

GenØk-Senter for Biosikkerhet
Desember 2016

Vår ref:2016_H_RX_003
Deres ref:2016/9453

Assessment of the application for renewal of DAS-59122-7 maize

Sent to

Norwegian Environment Agency

by

GenØk- Centre for Biosafety
December 2016

OPPSUMMERING

GenØk–Senter for Biosikkerhet, viser til høring av søknad om fornyelse av EFSA/GMO/RX/003 gjeldende for **DAS-59122-7 mais** som omfatter bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **DAS-59122-7 mais.**

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

- Adjuvans effekt av multiple cry proteiner.
- Glufosinat ammonium – helse-og-miljø fare ved bruk.

SUMMARY

We have assessed the documents available, and highlights in particular the following points for the current application for renewal of DAS-59122-7 maize:

- Adjuvance effect of multiple cry proteins
- Glufosinate ammonium – health and environmental related damage upon use.

ASSESSMENT OF THE APPLICATION FOR RENEWAL OF AUTHORISATION RELATED TO EFSA/GMO/RX/003

GenØk, as a National Competence Center for Biosafety, aims at providing independent, holistic and useful analysis of technical and scientific information/reasoning in order to assist authorities in the safety evaluation of biotechnologies proposed for use in the public sphere.

The information in this assessment is respectfully submitted for consideration in the evaluation of product safety and corresponding impact assessment of event **DAS-59122-7 maize**, setting out the risk of adverse effects on the environment and health, including other consequences of proposed release under the pertinent Norwegian regulations.

Main summary:

In our assessment of maize event DAS-59122-7 we find that data provided in the renewal of application on maize event DAS-59122-7 on social utility and sustainability is lacking. There is also a need for further investigation of whether Cry toxins Cry34Ab1 and Cry35Ab1 might have potential for non-target and adjuvant effects.

We therefore comment that the applicant has not provided the information required to perform an assessment of social utility and sustainability as required by the Norwegian Gene Technology Act (NGTA, Appendix 4) (1).

ASSESSMENT OF APPLICATION FOR RENEWAL OF AUTHORISATION OF EFSA/GMO/RX/003

Background

GenØk has previously assessed stacked events with maize event DAS-59122-7 in the following assessments:

- *EFSA/GMO/BE/2013/118: MON87427 x MON89034 x 1507 x MON88017 x 59122*
- *EFSA/GMO/NL/2011/92: 1507x59122xMON810xNK603*
- *EFSA/GMO/BE/2011/99: Bt11x59122xMIR604x1507xGA21*

Maize event DAS-59122-7 is not approved for any applications in Norway at present. In the EU, applications for approval for food, feed, import and processing expires on the 23. October 2017.

Maize event DAS-59122-7 has previously been evaluated by the Norwegian Biotechnology Advisory Board (2, 3) where questions on adjuvancy effects of the Cry proteins were raised to the Applicant, as well as effect on non-target organisms of these toxins. They asked the applicant to provide further details on the change in herbicide use that was expected and consequences for health and environment this potentially could have. The Norwegian Biotechnology Advisory Board recommended the Norwegian authorities to prohibit import due to the issues mentioned above and that it cannot be excluded that the Cry proteins have an adjuvancy effect that potentially can lead to allergic reactions. They also raised ethical concerns on the use of glufosinate ammonium, that has an effect on health and environment and that the assessment requirements in the NGTA (1) not is fulfilled when it comes to social utility and sustainability criteria.

A final assessment on DAS-59122-7 was made in 2014 by the Panel on Genetically Modified Organisms in the Norwegian Scientific Committee for Food Safety (4). They concluded that the gene modified maize event 59122 was nutritionally equivalent to conventional maize and that the presence of the Cry toxins Cry34Ab1 and Cry35Ab1 not were likely to introduce any allergic potential. They also concluded that with the intended usage, there was no risks to the environments in Norway.

The Norwegian Environment Agency has commented on another maize event with glufosinate ammonium tolerance (1507) to not have less social utility or contribute less to sustainable development than other maize (http://www.miljodirektoratet.no/no/Nyheter/Nyheter/2016/April/Anbefaler-ikke-forbud-mot-fem-genmodifiserte-planter/). However, they did not not lay down prohibitions against this kind of maize due to that.

The European Food Safety Authority (EFSA) Scientific Panel has made an opinion on the application EFSA/GMO/NL/2005/12 (5) for the placing on the market of maize 59122. The information provided by the applicant regarding maize 59122 were found to satisfy the scientific comments raised by Member States and the maize were found to be as safe as conventional maize.

ABOUT THE EVENT

Maize event DAS-59122-7 (also called "Herculex RW Rootworm Protection") is transformed by *Agrobacterium*-mediated gene transfer resulting in the expression of three protein products; Cry34Ab1 and Cry35Ab1 conferring resistance to certain Coleoptera species acting as pests, and PAT (Phosphinothricin acetyltransferase) providing tolerance to herbicides containing glufosinate ammonium.

ASSESSMENT FINDINGS

Bt-proteins

Event **DAS-59122-7 maize** combines two Bt-proteins named Cry34Ab1 and Cry35Ab1. These proteins, also called Bt-toxins work by giving the gene modified maize plants protection against certain Coleoptera insects. However, Bt-toxins also have the potential of non-target effects, and alternative modes of action for Cry toxins have been addressed previously (6-9).

Studies performed on non-target insects of Bt-proteins have documented that 30% of studies on predators and 57% of studies on parasitoids display negative effects to Cry1Ab transgenic insecticidal proteins (10). Further, Cry toxins and proteinase inhibitors have shown non-neutral effects on natural enemies, and seemingly more often negative than positive effects (11). A review by Hilbeck and Schmidt (9) on Bt-plants, found that half of the studies documented negative effects on tested invertebrates.

In addition, a review by van Frankenhuyzen (12) indicated that several Cry proteins exhibit activity outside of their target orders. This study also found that many Cry proteins had been tested with a very limited number of organisms: thus, activity outside of the target organisms of many Cry proteins may be undocumented because testing has not included sensitive organisms.

A quantitative review analysis based on 42 field experiments with GM plants showed that unsprayed fields of Bt-maize plants have significantly higher abundance of terrestrial non-target invertebrates than sprayed conventional fields (13). 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 (13).

Research on aquatic environments investigating potential impact of Bt-crops on aquatic invertebrates including *Daphnia magna* (6) and caddisflies (14) has also been performed. Douville et al. (15) presented data of the persistence of the *cry1Ab* transgene in aquatic environments: it persisted 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 (16). Impacts on soil microflora and fauna, including

earthworms (17) , mychorizzal fungi (18) and microarthropods in response to Cry endotoxins have also been reported (19-21). The significance of tri-trophic effects of accumulation, particularly of insecticidal Cry toxins (22, 23) is, however, yet to be firmly established.

In an experiment using broccoli plants containing Cry1Ac, Cry1C or both to investigate resistance development in a population of diamondback moths (*Plutella xylostella*), they found that when using stacked, similar Cry proteins, the resistance development in this population increased to both traits (24). Another group (25) later commented this on; suggesting that gene stacking might not be a solution to the development of resistance towards Cry proteins.

In one particular case with investigation of evolved resistance towards Cry34Ab1 and Cry35Ab1, which are present in the renewal of application of DAS-59122-7, no cross-resistance was detected when there was presence of yet another Bt-toxin (26). It has also been shown that the combination of two (or maybe more) insecticidal proteins against the same pest as target, is a tactic used to delay the resistance development towards either protein in the combination (27).

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 (28). We suggest that further studies should also include animals with immune-deficiencies and/or animals exposed to other stress agents simultaneously.

Adjuvancy effects

The potential adjuvancy of Cry proteins has previously been addressed by the GMO Panel of the Norwegian Scientific Committee for Food Safety (29). Scientific studies have shown that the Cry1Ac protein is highly immunogenic and has systemic and mucosal adjuvant effects (30). In the evaluation of another 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:

"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" (31).

The GMO Panel of the Norwegian Scientific Committee for Food Safety (29) also writes that:

"There are many knowledge gaps related to assessment of adjuvants. Most of the immunologic adjuvant experiments have been performed using Cry1Ac. Whether the other Cry proteins have similar adjuvant properties is unknown".

And;

"The possibility that Cry proteins might increase the permeability of the intestinal epithelium and thereby lead to "bystander" sensitization to strong allergens in the diet of genetically susceptible individuals cannot be completely excluded."

We also agree with these concerns and highlight them for the maize event DAS-59122-7.

Summary:

- Cry proteins might have potential for non-target effects.
- Pyramiding of Cry genes can delay resistance development of either of the proteins in the pyramide.
- As some Cry proteins have adjuvant effects, it can not be excluded that other Cry proteins have that also. This should be investigated.

Herbicides

The maize event DAS-59122-7 contains a PAT gene providing herbicide tolerance.

Herbicide use on GM plants

The combination of Bt-protein and herbicide tolerance (HT) gene is the most used combination of inserted genes when it comes to GM plants. In this case, maize event DAS-59122-7 is tolerant to the herbicide glufosinate ammonium, as well as expressing two Bt-proteins.

HT plants are sprayed with the actual herbicide, leaving the weed to die whereas the plant with the inserted gene will survive. However, the accumulation of herbicides inside plants is often not tested as part of the risk assessment of the HT plants.

In this application for renewal of DAS-59122-7 the maize is regulated by article 10 of EC regulation 396/2005 regarding "maximum residue levels (MRL) for the use of glufosinate in genetically modified maize containing pat gene…" The MRL for glufosinate ammonium is given in EC regulation 149/2008. It is unclear from the available literature if the gene modified maize is checked for level of glufosinate ammonium.

In some cases, data presented from feeding studies using HT plants have presented data where the HT plant material used, is not sprayed with the intended co-technology herbicide (32). In the renewal of application for DAS-59122-7 it is unclear from the data available if the plant material used for feeding studies is sprayed with the corresponding herbicide or not.

Another issue is the potential for accumulation of herbicides in the HT plants, including

metabolic pathways and metabolites of these. Recently, Bøhn et al. (33) 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 (34, 35). 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 ammonium tolerance

The event DAS-59122-7 maize contain the *pat* gene from *Streptomyces viridochromogenes* that confers tolerance to herbicides containing glufosinate-ammonium, a class of herbicides that are banned in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans. Glufosinate ammonium is harmful by inhalation, swallowing and by skin contact. Serious health risks may result from exposure over time. 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 (36-39) EFSA has concluded on the risk of glufosinate ammonium, as especially harmful to mammals (40).

CaMV Promoter

The 35S cauliflower mosaic virus (CaMV) promoter is commonly used to drive transgene expression in the genetically engineered (GE) crop plants that have been commercialized so far (41-43). Safety questions related to the use of the Cauliflower Mosaic Virus 35S promoter (P35S) in GM plants has recently been discussed in an article from Podevin and Du Jardin (44). In the article, the authors state that some P35S variants contain open reading frames (ORFs) that when expressed could lead to “unintended phenotypic changes”. Gene VI encodes the multifunctional P6 protein that can be divided into four domains (45). Functions of P6 include nuclear targeting (46), viral particle binding and assembly (47), si- and ds-RNA interference and interference suppression (48) and transcriptional transactivation (49, 50). The main debate when it comes to the use of this promoter is that it may not only be active in plants, but may confer activity with respect to gene expression in lower and higher vertebrates such as mammals and fish. Today there are reports that conclude that the 35S CaMV promoter is active in several eukaryotic cell lines after transfection (41, 43), as well as that the promoter is able to drive expression of a transgene in fish as demonstrated recently by Seternes et al (42). The potential risk when it comes to GM food/feed that contains the CaMV promoter may be unlikely but cannot be excluded.

Summary:

- DAS-59122-7 maize is tolerant to glufosinate ammonium. This herbicide is damaging to health and environment.
- Potential of accumulation of glufosinate ammonium should be considered for GM plants used in food and feed.
- DAS-59122-7 maize has a 35S CaMV promoter driving expression of one of the transgenes. This promoter is shown active in plant as well as mammalian cells and that some variants have ORFs.

INFORMATION RELEVANT FOR THE GENETIC MODIFICATION
The assessment is based on the documentation that is available on EFSAs webpage GMO EFSAnet.

Molecular characterization

- Maize event 59122 was transformed by *Agrobacterium tumefaciens* mediated gene transfer technology and expresses *CRY34Ab1*, *CRY35Ab1* and *PAT* proteins.
- Maize 59122 has no marker genes for antibiotic resistance.
- In this maize line, the *Pat*-gene that confers tolerance to glyphosinate-ammoniuim herbicide, was used as a selectable marker.
- The CaMV 35S promoter from cauliflower mosaic virus regulates the expression of pat.
- Southern analysis in addition to PCR and DNA sequencing data demonstrated that maize 59122 contains a single insert of the T-DNA and that no vector backbone sequences were detected. The probes used in this application ranged in size from 317-1081 bp.
- The quality of the data provided is in general ok, however some of the probes used in the application are oversized and some of the Southern-blot gel pictures are of sub-par quality, which makes it difficult to draw conclusions.

Information on the expression of the inserted/modified sequences
The expression of the proteins Cry34Ab1 and Cry35Ab1 was analysed in maize plants at different developmental stages using Enzyme Linked Immunosorbent Assay (ELISA). The proteins were expressed in all parts of the plant (and grain).
Level of PAT was detected in all maize tissues tested (not specified in Part II of the Summary of the Application) and found in levels below detection or at very low levels.

Toxicological assessment
All three proteins expressed has previously been evaluated as safe for human food and animal feed use by EFSA through:

- Previous history of safe use
- Mode of action
- Specificity
- Absence of toxicity to mammals or fast growing species
- Biochemical characterization of the proteins and lack of aminoacid sequence similarity to known toxins.
- Lack of resistance to proteolysis and lack of stability when heated.

It is not clear if the maize used for the 90-day oral toxicity study or in the 42 days poultry study was sprayed with the herbicide intended to be used on the genetically modified maize.

Summary:

- Expression of Cry34Ab1, Cry35Ab1 and Pat were found in all analysed tissues.

- Safety data are based on previous characteristics and evaluations on history of safe use and biochemical characteristics.
- From the application, it is unclear if spraying with herbicide is included in the feeding experiments with DAS-59122.

Allergenicity assessment
Based on the principle of weight of evidence, results for all three proteins point to that there is no significant risk of the proteins being allergenic.

For the issue of adjuvancy of Cry proteins: see page 10 for further comments.

SOCIAL UTILITY AND SUSTAINABILITY ASPECTS
In addition to the EU regulatory framework for GMO assessment, an impact assessment in Norway follows the Norwegian Gene Technology Act (NGTA) (1). In accordance with the aim of the NGTA, production and use of the GMO shall take place in an ethically and socially justifiable way, under the principle of 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*". These issues are further elaborated in the regulations relating to impact assessment pursuant to the NGTA, section 17 and its annex 4. Moreover, new European legislation on GMOs allows 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 (51). Additionally, in recent years there has been an increase in attention within academic as well as policy spheres to include broader aspects in the assessment of new and emerging (bio)technologies beyond human and environmental health, such as sustainability, benefit for society and ethical considerations (52-57).

The Applicant has not provided relevant information that allows an evaluation of the issues laid down in the aim of the Act, regarding ethical justification, social utility or the contribution to sustainable development of the GMO. Given this lack of necessary information for such an evaluation, the Applicant has not demonstrated a benefit to the community and a contribution to sustainable development from the use of maize 59122.

In the following, we identify areas that are relevant to consider in order to assess the criteria of social utility, ethical justifiability and the contribution to sustainability and highlight information that is missing from the Applicant.

Impacts in producer countries
As already stated, the Applicant does not provide data relevant for an environmental risk assessment of 59122, 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. Importantly, 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. Hence, a proper evaluation of potential impacts that are relevant for sustainability is lacking, and sufficient information relevant for the ERA and socio economic impacts assessment in these agricultural contexts needs to be provided. This should include information from an ERA concerning impacts on cultivation, management and harvesting stages, as well as the post-market environmental monitoring in the producing country. With regard to potential socio-economic impacts in the producer country or countries, published reviews on sustainability-relevant aspects (e.g. impacts among poor and/or small-scale farmers in developing countries, share of the benefits among sectors of the society) indicate that these effects have been very complex, mixed and dependent on the agronomic, socio-economic and institutional settings where the technology has been introduced (58). The applicant does not provide any references to the extensive literature concerning the socio-economic aspects related to the cultivation (and to a much lesser extend, the use) of GM maize.

Assessment of alternatives
It is also important to evaluate whether alternative options may achieve the same outcomes in a safer and ethically justified way. This relates to the increased trend to anticipate impacts and reflect on underlying values, assumptions, norms and beliefs within research and policy of science and innovation (55, 59) 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. Indeed, in order to evaluate whether 59122 maize contributes to social utility, it is important to consider current and future demand for this GM-maize product for food, feed and processing purposes in Norway and to what extent this demand is/can be satisfied by existing sources. By 2013 around 32% of all maize grown worldwide are GM[1], hence alternatives to GM maize can meet the present demand for maize in Norway. The maize event 59122 does not contain genes that codes for proteins that is of specific need in Norwegian food, feed, or industrial processes.

About the use of glufosinate-ammonium
Maize event 59122 is tolerant to glufosinate-ammonium that is banned for use in Norway. While it is understood that the Applicant has not applied for deliberate release of 59122 maize in Norway, the acceptance of a product in which the intended use involves the use of a product banned in Norway, raises questions to the criteria of sustainability as laid out in the NGTA. Indeed, within the NGTA, the aim to assess the contribution to sustainable development is not limited to Norway, but there is significant emphasis to consider the impacts and consequences for producing countries from which Norway imports food and feed as well. Specifically, this issue is relevant particularly in the revised guidelines for impact assessment pursuant to the Act of 2005 Section 17, "*Other consequences of the production and use of genetically modified organisms"* points 2 and 3, "*ethical considerations that may arise in connection with the use of the genetically modified organism(s)*», and "*any favorable or unfavorable social consequences that may arise from the use of the genetically modified organism(s)*", respectively.

[1] http://www.gmo-compass.org/eng/agri_biotechnology/gmo_planting/257.global_gm_planting_2013.html

Final 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 social utility of 59122 maize and its contribution to sustainable development. The information provided by the Applicant must be relevant for the agricultural context in the producing country/countries, and for Norway as a potential importing country. The information should include issues such as:

- changes in pesticide use,
- development of pest resistance in target populations,
- impacts on non-target organisms,
- potential for adjuvancy effects
- potential for gene flow
- possible impacts among poor and/or small-scale farmers in producing countries,
- share of the benefits among sectors of the society, and
- meeting a need among consumers or industry.

Furthermore, 59122 maize is tolerant to glufosinate-ammonium which is banned for use in Norway due to health and environmental concerns. How the use of this herbicide contribute to sustainable development in the producing country needs therefore to be demonstrated by the applicant. Moreover, the applicant does not attempt to identify ethical implications, nor demonstrate a benefit to the community in Norway or in the producing country from the use 59122 maize and does therefore not provide sufficient information as required by the NGTA.

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