GenØk - Centre for Biosafety

Impact assessment of maize hybrid MON 89034 x NK603 from Monsanto and Dow AgroSciences (EFSA/GMO/NL/2009/72)

With Conclusion in Norwegian

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KONKLUSJON

Genøk –Senter for Biosikkerhet viser til brev fra Direktoratet for naturforvaltning (DN) angående høring relatert til maisplanten MON 89034 x NK603 for bruksområdene import, prosessering, mat og fôr.

Maisplanten MON 89034 x NK603 er en stablet hybrid (”multistack”) ulike pesticid-kodende gener (Bt-toksiner) innebygd. I tillegg er den tolerant for sprøytemidler glyfosat.

Informasjonen som er tilgjengelig fra søker er ikke tilstrekkelig for uavhengig evaluering av søknaden. Det foreligger ingen resultater fra analyser eller detaljerte forsøksoppsett til oppklaring av DNA sekvens, lokalisering av transgenet i maisgenomet, protein uttrykk, toksikologiske/immunologiske effekter eller foringsforsøk i relevante dyremodeller. En av morplantene (NK603) viser helserelevante og signifikante negative effekter i rotteforsøk etter re-analysering av søkers egne foringsforsøk. Det er ikke opplyst hvorvidt søker har frigitt frø fra den genetisk modifiserte planten og relevante ikke-GMO kontroll planter. Dette er nødvendig for at fri og uavhengig forskning med denne planten skal være mulig.

Stablede hybridplanter har generelt en mer kompleks genetisk sammensetning og derfor større potensial for opp- og nedregulering av plantens egne gener. Derfor burde de gjennomgå grundig testing før eventuell markedsadgang. GenØk mener det ikke er faglig velbegrunnet å godkjenne stablede hybrider basert på at foreldrelinjene, hver for seg, er godkjent.

Det kan ikke utelukkes at gruppen av de uttrykte toksinene cry1A.105, cry2Ab2 og cry1F i planten kan gi spesifikke immunogene effekter eller adjuvanseffekter (fremming av immunreaksjoner mot andre stoffer) hos pattedyr og mennesker.

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnssnytte og etiske aspekter som forutsettes anvendt i den norske genteknologiloven. I denne sammenheng er det viktig å få dokumentert om den omsøkte planten fører til mindre bruk av sprøytemidler, samt erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter hos bønder som dyrker den. Denne type dokumentasjon er ikke vedlagt søknad om omsetting av MON 89034 x NK603.

Basert på manglende uavhengige studier og data tilgjengelig vi ønsker å påpeke at det er kunnskapshull relatert til risiko for helse og miljø ved MON 89034 x NK603. Disse kunnskapshullene er spesielt relatert til usikkerhet ved effekter som kan oppstå på grunn av kombinasjonen eller synergistiske effekter av de innsatte genene og viser til at en bør bruke føre-var prinsippet og ikke godkjenne bruk i Norge.

ABOUT THE PLANT

The parent lines contribute the following transgenes: *cry1A.105*, *cry2Ab2* and *cry1F* from MON89034 and a C4-EPSPS gene from NK603. These genes give the plant resistance to insect herbivores and tolerance to the herbicide glyphosate.

In general the parts of the application made available for comments lack details about every experiment performed by the applicant. For instance, the applicant makes references to expression studies performed in USA in 2006, but there are no data presented from these studies (section 3). This makes it impossible to evaluate the relevance and statistical power of these analyses. Also, in the application summary the applicant states under section D6: "Since the insert present in MON 89034 x NK603 corresponds to that of the parental lines, the insertions and the 5´and 3´flanking sequences are likely to have been conserved in this hybrid". The applicant should provide extensive sequence data on both the constructs and flanking sequences in the final hybrid and in subsequent generations of offspring in order to investigate the stability of the insert. The same applies to all other points in the application under section D (Information relating to the GM plant). This information must be made available to the public and scientific communities.

There is no scientific literature available on the genetic construct and genetic stability of the stacked event in question in order to make an appropriate scientific evaluation. According to the applicant, MON 89034 x NK603 has been field tested, therefore the applicant should therefore provide information on the stability of the insert over multiple generations as well as compositional data and expression analyses over all growing seasons.

The same critique is valid for all points under sections C and D

BT PROTEINS AND IMMUNE EFFECTS

There are no data available from the scientific literature on the genetic stability, transgene expression products or immuno-toxicological effects, or cross resistance effects of the MON 89034 x NK603 stacked event.

Published mouse experiments have demonstrated that Cry1Ac (member of the Cry1A class of endotoxins) raises specific immune reactions, and also possesses adjuvant properties by increasing the immunogenicity of proteins intermixed with feed products (Moreno-Fierros et al. 2003; Vazquez et al. 1999; Vazquez-Padron et al. 1999; Vazquez-Padron et al. 2000), (Rojas-Hernandez et al. 2004). This may result in increased immunological and allergic responses. In other words, the likelihood of immunological and allergic responses increases if Cry1Ac is administered together with a dietary antigen/allergen. Published data also suggest that Cry proteins may inhibit development of mucosally induced suppressive immune mechanisms referred to as "oral tolerance" against innocuous food proteins (Brandtzaeg 2007). In investigations with Cry1Ab (another Cry1A class protein) (Guimaraes et al. 2008) did not find a similar type of adjuvant effect elicited against peanut proteins as with Cry1Ac, yet instead found evidence of Cry1Ab acting as an adjuvant leading to early phase production of leukotrienes and increased Th2 and Th17-cytokine production in branchoalveolar lavage fluids after airway exposure. The implication of possible effects of Cry1Ab to produce allergen-induced cytokine responses is an area of investigation warrant further inquiry.

There are a number of difficulties when it comes to studies of food allergies in humans. The frequency of food allergy in the human population is about 2 % in adults and about 5 % in children (EFSA Opinion 2004) and seems to require a genetic predisposition. Whether the

possibility/risk of food allergy increases with the presence of intestinal localized Cry proteins is not known. Therefore, one should not expect a high incidence of adverse effects in the general population due to ingestion of food containing adjuvants enhancing the development of allergy. But the use of transgenic feed maize containing multiple Cry proteins, brings up a concern whether there will be a higher incidence rate for food allergy. In addition, since the Cry proteins possess adjuvant activity there may be enhanced inflammatory processes.

Combinatorial or synergistic effects of recombinant proteins acting as adjuvants to immunostimulatory effects, or as potential allergens are areas of important coming scientific inquiry.

NON-TARGET EFFECTS AND EFFECTS ON BIODIVERSITY FROM BT-PROTEINS

In two meta-analyses of published studies on non-target effects of Bt proteins in insects, (Lövei and Arpaia 2005) documented that 30% of studies on predators and 57% of studies on parasitoids display negative effects to Cry1Ab transgenic insecticidal proteins. A review by (Hilbeck and Schmidt 2006) on various Bt-plants found 50% of studies documenting negative effects on tested invertebrates.

Another quantitative review by (Marvier et al. 2007) suggested a reduction in non-target biodiversity in some classes of invertebrates for GM (Bt) cotton fields vs. non-pesticide controls, yet found little reductions in biodiversity in others.

More recent research on aquatic environments has sparked intense interest in the impact of Bt-crops on aquatic invertebrates *Daphnia magna* (Bøhn et al. 2008), and caddisflies (Rosi-Marshall et al. 2007). These publications warrant future study, given the potential load of novel target proteins that may end up in agricultural runoff and end up in aquatic environments. Further, (Douville et al. 2007) present evidence of the persistence of the transgenic insecticidal protein Cry1Ab in aquatic environments and suggest that that sustained release of this potently bioactive compound from Bt maize production could result in negative impact on aquatic biodiversity.

Impacts on soil microflora and fauna, including earthworms (Zwahlen et al. 2003), mychorizzal fungi (Castaldini et al. 2005) and microarthropods in response to Cry endotoxins have also been reported (Griffiths et al. 2006; Wandeler et al. 2002).

The significance of tritrophic effects of accumulation, particularly of insecticidal Cry toxins (Harwood et al. 2006; Obrist et al. 2006) is, however, yet to be firmly established. It has been demonstrated that subchronic dosages of Cry proteins may affect both foraging behavior and learning ability in non-target bees (Ramirez-Romero et al. 2008), and may have indirect effects on recipient populations, and, given the key-stone role of bees as pollinators, on both primary production and on entire food-webs.

ANIMAL AND HUMAN HEALTH

A recent publication by (Dona and Arvanitoyannis 2009) reviews the potential health implications of GM foods for humans and animals, including incidences and effects of increased immunogenicity, amounts of anti-nutrients, possible pleiotropic and epigenetic effects, including possible reproductive and developmental toxicity. They conclude that while there is strong evidence for health concerns on many fronts, exposure duration have not been long enough to uncover important effects. Studies should also include subjects with immunodeficiency or exposed to other stress agents.

Feeding studies

Spiroux de Vendomois et al. (2009) independently evaluated the methodology and raw data of a comparative NK603/non GM isogenic maize rat feeding study, conducted by the applicant, Monsanto and used as scientific evidence of safety. The researchers draw 3 broad and scientifically important conclusions:

1) The sample sizes used in the trials were insufficient to detect all but very acute differences (small sample sizes make it less likely to detect effects when they exist), and lacked sufficient statistical power to draw relevant conclusions,

2) Study design and statistical methods employed would often fail to identify relevant effects,

3) Using statistical methods designed to detect different types of effects, the researchers found significant dose and sex dependent (male more susceptible than females) side effects linked with the consumption of the NK603 that was not reported by the producer.

Although the authors call for a repeat of the experiment, and caution that the deficiencies in the study design make it difficult to draw definitive conclusions of toxicity, the authors state in their conclusion that "[O]ur data presented here strongly recommend that additional long-term (up to 2 years) animal feeding studies be performed…" (Ibid p. 718).

Bt Cry toxins

Once more, no information about the stacked event in question is available, but some studies have been performed on parental lines.

Immunological effects have largely focused on potential allergenicity of GMOs, rather than broader suites of immunogenic or toxicological responses. Inhalation studies, rather than oral toxicity are also largely missing from the scientific literature (yet see Spiroux de Vendomois et al.,2009 above). One study by (Kroghsbo et al. 2008) found increased antigen-specific antibody response to Bt toxin and PHA-E lectin in a 28 and 90-day feeding study of Wistar rats.

A study by (Schroder et al. 2007) found a significant difference in white blood cell count and reduced kidney weight among male rats in a 90 day feeding trial with Bt rice.

A team of Austrian researchers conducted feeding trials with a stacked Bt maize event (NK603 x Mon810) and found significant effects vs. non-Bt maize. Along with reports of kidney toxicity, the authors concluded that "multi-generation studies, especially based on the [reproductive assessment by continuous breeding (RACB)] design are well suited to reveal differences between feeds. The RACB trial showed time related negative reproductive effects of the GM maize under the given experimental conditions. The RACB trial with its specific design with the repeated use of the parental generation is a demanding biological factor for the maternal organism" (p. 4 (Velimirov et al. 2008).

In a 2008 feeding trial on mice with MON810 Bt maize, (Finamore et al. 2008) concluded: "induced alterations in intestinal and peripheral immune response of weaning and old mice. Although the significance of these data remains to be clarified to establish whether these alterations reflect significant immune dysfunctions, these results suggest the importance of considering the gut and peripheral immune response to the whole GM crop, as well as the age, in the GMO safety evaluation" (Ibid, p. 11537).

COMBINATORIAL AND/OR SYNERGISTIC INDIRECT EFFECT WITH STACKED TRAITS IN TRANSGENIC PLANTS

The recent development and commercialization of GMOs with multiple transgenic traits have prompted an interest in the possible combinatorial and/or synergistic effects that may produce unintended and undesirable changes to endogenous or introduced traits and functions. The indirect effects of such changes may impact the sustainable development of future GMOs, and come with high uncertainty with regard to other unintended effects that will need to be monitored in the future.

In the case of simultaneous exposure to different classes of Cry proteins introduced in tandem, despite different modes of insecticidal activity, (Tabashnik et al. 2009) found evidence of cross reactivity among "pyramided" (stacked events) of Cry1Ac and Cry2B endotoxins in transgenic cotton. The cross reactivity led to higher rates of resistance evolution in pink bollworm, Pectinophora *gossypiella,* in a laboratory setting. Their results suggest that in the case of different Cry protein species, cross reactivity between them may confer increased rates of insect resistance that would alter the efficacy and perhaps biological activity of the GMO.

Then (2009) reviews and discusses the evidence for changes in activity and specificity of Bt proteins dependent on synergisitic interactions with extrinsic features. Such changes may critically influence the bioactivity and hence the potential for unintended effects.

Combinatorial, synergistic effects must be carefully considered in the development and risk assessment of stacked event GMOs with respect to the implications on biodiversity and evolutionary consequences for crop genetic diversity. This will be an important area of investigation for risk research, as multi-trait (stacked) GMOs are poised to replace the current generations of GM crops used in global agriculture. More research in this area is needed.

The issue of combinatorial and/or synergistic effect of transgene proteins either with endogenous host proteins or with other inserted GM traits (e.g. "stacked" events) is an area of nascent scientific inquiry. Several studies point towards extrinsic factors that may modulate Cry (Bt) efficacy and specificity. For example (Broderick et al. 2006; Broderick et al. 2009) found that midgut bacterial presence was required for Cry1Ab insecticidal activity in gypsy moth (*Lymantria dispar*) only suggesting the intestinal microflora may modulate toxicity in certain target Lepidopteran insect species. Further, research by Soberon et al (2007) suggests that structural changes to the engineered Cry1Ab protein in cotton may lack important oligomerization features essential to toxin efficacy against *P. gossypiella*.

HERBICIDES

MON 89034 x NK603 tolerates higher doses of the herbicide glyphosate compared to weed plants.

In recent years glyphosate has received more risk-related attention due to negative effects on both aquatic and terrestrial ecosystems (Blackburn and Boutin 2003; Ono et al. 2002; Solomon and Thompson 2003). Studies in animals and cell cultures indicate possible health effects in rodents, fish and humans. Glyphosate given in the feed to pregnant female rats resulted in higher embryonic mortality and aberrations in the skeleton (Dallegrave et al. 2003). Nile-tilapia (*Oreochromis niloticus)* fed sublethal concentration of Roundup (active ingredient: glyphosate) resulted in a number of different histopathological changes in organs (Jiraungkoorskul et al. 2003). Experiments with sea urchins exposed to Roundup influenced early cell divisions (Marc et al. 2002), effects that have relevance to potential health effects in many eukaryotic organisms, including domestic animals and humans. Exposure to Roundup affected the CDK1/CyclinB regulator which is nearly identical in sea urchins and humans. Glyphosate has also been shown to negatively affect the differentiation of nerve cells (Axelrad et al. 2003). In human placenta cells, Roundup is more toxic than the active ingredient glyphosate (Richard et al. 2005). The authors concluded that additional components of Roundup increase the biological availability and accumulation in organisms. From the US, the use of *epsps*-transgenic plants has led to increased use of glyphosate compared to conventional plants (Benbrook 2003).

PRECAUTIONARY APPROACH TO RISK ASSESSMENT

The Precautionary principle requires commitment to the idea that full scientific proof of a causal link between a potentially damaging operation and a long term environmental impact is not required to take action in order to avoid negative effects on health and the environment. Due to the lack of information available in the scientific literature genetic stability, expression of inserted proteins or immune effects as well as the stacked event of the MON 89034 x NK603, we find that these uncertainties warrant further research and advice the DN to apply the Precautionary principle and deny the marketing until more scientific understanding has been published.

AVAILABLE INFORMATION FOR RISK ASSESSMENT EVALUATION

This evaluation is for the most part based on the applicants own submitted information. The directly relevant peer-reviewed literature is very limited but we have tried to extract relevant indirect information from the peer-reviewed literature.

All product-related safety testing should have an independent and unbiased character. This goes both for the production of data for risk assessment, and for the evaluation of those data. If a company would suffer additional costs, reduced incomes or delayed entrance into the marked, we claim that they are vulnerable to being biased in their hypothesis, study design, presentation and interpretation of the data.

The aforementioned study by Spiroux de Vendomois et al. (2009), along with other studies that have investigated producer safety data (Marvier, 2002; vom Saal and Hughes, 2005) highlights the need for independent evaluation of safety studies. **We therefore request that mechanisms become available that allow access of this data to independent scientists and biostatisticians to verify the scientific soundness of methods and statistics employed**, as such independent evaluation is essential to maintaining rigorous standards expected in scientific practice.

ON CONFIDENTIALITY OF INFORMATION

The documentation accompanying GMO applications may be problematic for four reasons. The first problem regards transparency and confidentiality. Some of this information is available on the Net, through the European Food Safety Authority (EFSA), but varying parts of it is confidential. Although some information might be regarded as CBI, we cannot see any reason at all that feeding studies, their methods and all primary data/results can be protected under CBI. In that case CBI becomes a justification for protecting important information that can help assess the risk of products to human health, the environment, or food security.

The problem of confidentiality that is linked to the documentation provided by the GMO applications has several implications. Access to peer-reviewed quality data is essential for a "science-based" risk assessment. In order to gain regulatory approval, commercial developers of GMOs often submit their own test results to document the expected behavior of the GMO and its products in the exposed system, and hence, its safety. In this case, we did not find any experimental data on the safety of MON 89034 x NK603 available in the peer-reviewed literature. The available documentation is supplied with references, but a substantial part of these references point back to the research departments of the applicant itself, are considered confidential business information and therefore not accessible. Another problem is partly particular to the Norwegian situation, namely that important aspects are lacking. Most apparent is of course the lack of information about sustainable development and societal utility.

SUSTAINABILITY

In addition to the EU regulatory framework for GMO assessment, impact assessment in Norway follows the Norwegian gene technology act, which states that "in deciding whether or not to grant the application, significant emphasis shall also be placed on whether the deliberate release represent a benefit to the community and a contribution to sustainable development" hence it is obvious that, for the Norwegian authorities, that contribution to sustainable development should be assessed together with an evaluation of the societal utility in applications of use and release of GMOs. With the purpose to guide political decisions concerning GMO and the aim of the gene technology act, Norwegian authorities has with the basis in the biotechnology advisory boards discussion paper: "Sustainability, benefit to the community and ethics in the assessment of genetically modified organisms" (2003) elaborated in the impact assessment regulations annex 4 several questions (see box 1).

Box 1. Appendix 4 to the Norwegian Impact assessment regulation

1. Global impacts
2. Ecological limits
3. Basic human needs
4. Distribution between generations
5. Distribution between rich and poor countries
6. Economic growth

With regard to questions ***1: global impacts*** and ***2: ecological limits*** the performed risk assessment by the applicants can be used to consider whether the issues listed in Table 1 have been assessed. However, as illustrated in table 1 does this issues also cover more broad aspects which there are no information attached in the application (Rosendal and Myhr 2009). With regard to questions ***3: basic human needs, 4: distribution between generations, 5: distribution between rich and poor countries***, and ***6: economic growth***, there is no information within the documents that follow the application.

Table 1. Information relevant for checklist on sustainability.

Checklist in appendix 4	**Relevant information that can be found in applications**	**Information lacking in applications**
Global impacts	Persistence, invasiveness, possible population and fitness changes introduced in the GMP. Potential for gene transfer. Interaction between GMP and target organism. Interactions between GMO and non-target organisms.	Changes in biogeochemical processes. Changed due to cultivation patterns. Effects on water and energy balance. Latency / cumulative effects. Impacts on socio-ecological relationships.
Ecological limits		
Basic human needs	Benefits for health. Toxicity and allergenicity.	Latency / cumulative effects. Food security issues.
Distribution between generations	Not found	Latency / cumulative effects. Influence on scientific developments. Trade-off between utility and risk.
Distribution between rich and poorer countries	Not found	Adequacy for meeting problems in poor countries and especially for small-scale farmers.
Economic growth	Not found	Latency/ cumulative effects. Trade off between short-term economic growth versus potential long term adverse effects.

The Norwegian Gene Technology Act, with its clauses on societal utility and sustainable development, comes into play with a view also to health and environmental effects in other countries, such as where GMOs are grown, often in Third World countries. For instance, most Bt maize has been developed and tested in the US and it is difficult to translate and extrapolate risk assessment results on the toxicity of Bt maize to human and non-target organisms to other countries because there are great differences between; regional growing environments, scales of farm fields, crop management practices, local/ regional target and

non-target species considered most important in the agro-ecosystem, interactions between cultivated crops, and surrounding biodiversity. Toxicity and environmental impact data on other species (e.g. regionally appropriate non target insects, including other non-domesticated herbivores) and regional environments (local growing regions) would be needed to accurately determine toxicity and environmental impacts to local fauna of the five different Bt toxins and its degradation products, i.e. resulting from ingestion by herbivores and decomposition in the soil of plant material and root exudates. Even for target pest species from different countries or regions, sensitivities to expressed Bt toxins vary widely. Hence it cannot be expected that the same species-specific and even population-specific sensitivity to Bt toxins will apply between different environments and across continents. Local non target species like butterflies of conservation concern and heritage value may therefore be at risk.

Hence, Norwegian authorities should contact the applicant directly and require the required information in accordance with the Norwegian Gene Technology Act.

SOCIETAL UTILITY

The concept of societal utility is found in the Gene Technology Act §10. Societal utility is a complicated concept that may have multiple meanings. The assessment of societal utility can be evaluated to a) the products properties, and b) the development and use of the product, and has elaborated the following questions to be addressed;

The products properties;

- Is there a need for this product?
- May the product solve or contribute to solve a societal problem?
- Is the product better than equivalent products on the market?
- Are there any alternative products that may solve or contribute to solve the societal problem in questions?

The development and use of the product;

- Does it help to create new opportunities?
- Does it help to create new opportunities in urban areas?
- Does it help to create new opportunities in other countries?
- Does it entail problems for existing production that need to be conserved?
- Does it entail problems for existing production in other countries?

Maize is not very important in Norway as food, but is of high relevance to the feed industry. Although it at present is not as difficult for Norwegian importers to get maize that is free of GM, this may change in the future. The applicant of MON 89034 x NK603 argues that consumption is safe; this is supported in general by the use of other Bt maize varieties. The majority of feeding studies support the claims that the GM maize is as safe as the non-GM counterpart, although there are uncertainties as described in the beginning of this document. Another issue of importance is that the pests that the GM maize is resistant against, is not a relevant problem in Norway; hence the GM plants in question cannot solve problems in Norwegian agriculture. In other parts of the world where pests are a major problem, the use of MON 89034 x NK603 may hold promises for environmental benefits to agriculture by increasing yield. Given the use of pesticides, a reduction of inputs of pesticides is also possible, leading to reduced exposure to farmers. However, the issue is more complex due to employment of resistance management, potential resistance development among pests and with regard to the usefulness of Bt toxins against the most important pests. The cultivation of GM plants in general is also causing problems with regard to co-existence. For instance Binimelis et al. (2008) have investigated consequences on co-existence of Bt-maize in Spain

among small-scale farmer and has found that co-existence is very difficult and that farmers in some areas has given up growing non-GM maize. In this context it is important to acknowledge that cultural concerns may be more significant than the functional utility, which has been highlighted with the debate concerning effects on Monarch butterflies and landrace corn in Mexico.

Conclusion

Based on the above, and with special attention on the independent evaluation of safety data with NK603, confidence in the safety of this maize variety (MON 89034 x 1507 x NK603) is scientifically unjustified at this time. Further evidence of lack of harm, including follow up feeding studies of longer duration and higher statistical power are needed.

Therefore, in our assessment of MON 89034 x NK603 we conclude that based on the available data, including the safety data supplied by the producer, is insufficient and equivocal in its proof of lack of toxicological affects on mammalian health. We find that these effects may be biologically significant and warrant future study before claims of lack of harm can be scientifically established.

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