GenØk - Centre for Biosafety

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Assessment of the technical dossier submitted under EFSA/GMO/NL/2011/69 for approval of transgenic potato event AV43-6-G7 by AVEBE U.A.

Submitted to

Direktoratet for Naturforvaltning

by

Centre for Biosafety – GenØk
and
Center for Integrated Research in Biosafety
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Innspill til offentlig høring av søknad EFSA/GMO/NL/2011/69

Konklusjon på norsk

GenØk har gått nøye gjennom de dokumenter som er sent inn av søker og som utgjør grunnlaget for søkers argumentasjon om at GM potet AV43-6-G7 er like sikker som konvensjonell potet, også for bruk som for eller mat.
Etter vår mening er det flere svakheter og mangler ved den fremlagte dokumentasjonen, metodene brukt i forsøkene samt begrepsformuleringer. Dette inkluderer mangel på nødvendig informasjon om potensielle utilsiktede effekter, uriktige antagelser og mangel på informasjon angående produktets samfunnsnytte og bærekraftighet, som gjør at søknaden etter vår mening, ikke oppfyller de krav som stilles i norsk lovgiving for godkjenning av import til og bruk i Norge.

Vi har lagt ved en engelskspråklig detaljert gjennomgang av søknaden og de tekniske bakgrunnsdokumentene, hvor vi påpeker mangler og kommer med spesifikke anbefalinger. Hovedfunnene er imidlertid gjengitt her i denne konklusjonen på norsk.

1. For å kunne karakterisere risiko av GM potet innblandet i Norsk diett, er det helt nødvendig med informasjon om hvordan en eksponering av produktet kan tenkes å foregå, i hvilken mengde og i hvilken form dette kan skje. Her har søker ikke gitt tilstrekkelig informasjon om noe av dette, slik at risiko ikke kan vurderes skikkelig.

2. Søker har ikke gitt overbevisende vitenskapelige bevis for å ha identifisert eller analysert såkalt off-target (ikke målgruppe) effekter av det unike dobbelt trådet RNA (dsRNA) eller nye uttrykt i AV43-6-G7 potet. De har ikke karakterisert fusjonsproteiner tilstrekkelig. Det samme gjelder for metabolske forandringer.

3. Søker har ikke undersøkt eventuell produksjon av nye små peptider forårsaket av lavnivå uttrykk av dsRNA. Søker har bare argumentert for at disse ikke eksisterer, men dette argumentet mangler vitenskapelige bevis. Den molekylære karakteriseringen er dermed utilstrekkelig for å kunne konkludere med at det ikke dannes nye unike protein-baserte farer (hazards).

4. Søker har i sin "oral toxicity test" funnet flere statisk signifikante effekter som søker ikke mener er relevant blant annet fordi de bare oppstår i dyr av ett kjønn og ikke har en klar lineær dose respons effekt (effekten øker ikke med tid og dose). Denne konklusjonen mener vi ikke er korrekt. I artikkler fra 2009 og 2011 har Seralini og medforfatterne diskutert kjønnsrelaterte og ikke-lineære tegn på toksisitet, og de henviser til eksempler på dokumenterte kjønnsforskjeller og til at hormonforstyrrende effekter ikke nødvendigvis har en slik lineær sammenheng, men ofte U eller J formede kurver. Slike effekter vil kunne oppstå ved enkelte tidspunkter avhengig av alder og eksponering av testindividene.

Derfor mener vi at statistisk signifikante forskjeller ikke kan avskrives som irrelevante på grunnlag av at de ikke forekommer i begge kjønn eller har en lineær sammenheng mellom dose respons (Seralini et al., 2009; Seralini et al., 2011).

5. Når man ser på den Norske genteknologiloven, appendix 4 del V, er det høyst tvilsomt om AV43-6-G7 oppfyller de krav som stilles i loven om samfunnsnytte. Etter vårt skjønn er det ingen fordel for norske forbrukere, bønder eller produsenter å benytte seg av denne amylose-reduserte genmodifiserte poteten når det finnes ikke-GM amylose reduserte potetvarianter på markedet. Det er også høyst tvilsomt om AV43-6-G7 er et positivt bidrag til bærekraftig utvikling.

Hovedkonklusjon og anbefalinger

Vi har i vår gjennomgang funnet flere svakheter av begrepsmessig art, mangel på informasjon, feilaktige konklusjoner og mangelfulle empiriske data som hver for seg og til sammen ikke støtter søkers påstand om sikker bruk, samfunnsnytte og bærekraftighet av AV43-6-G7. Søker har ikke fremskaffet noe av den informasjonen som er nødvendig for å kunne vurdere samfunnsnytte og bærekraftighet, noe som er påkrevd i den norske genteknologiloven for godkjenning i Norge. Disse manglene gjør at vi mener at denne søknaden er ufullstendig i nåværende form. Vi anbefaler derfor å avslå søknaden samt at en ny søknad bare bør vurderes om søker har adressert de mangler vi har belyst.

Summary of the assessment of the technical dossier related to EFSA/GMO/NL/2011/69

As a designated National Competence Center for Biosafety, our mission at GenØk in advice giving is to provide 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 following information is respectfully submitted for consideration in the evaluation of product safety and corresponding impact assessment of event AV43-6-G7, setting out the risk of adverse effects on the environment and health, including other consequences of proposed release under the pertinent Norwegian regulations.

This submission is structured to address specific provisions for an impact assessment required under the Norwegian Gene Technology Act of April 1993, focusing on the requirements in Appendix 2 - Principles for environmental risk assessment pursuant to sections 13-16 of the regulations, and Appendix 4 - Evaluation of ethical considerations, sustainability and benefit to society, cf section 17 of the "Regulations relating to impact assessment pursuant to the Gene Technology Act" of December 2005, pursuant to section 11 cf section 8. The information presented here may be applicable to more than one provision in different appendices. We focused our critique to address the information needs under the relevant provisions that relate to our particular area of competence in biotechnology assessment as comprehensively as possible. Lack of commentary on our part towards any information under consideration should not be interpreted as specific endorsement of that information.

This submission was built in large part using the **Biosafety Assessment Tool** (https://bat.genok.org/bat/) produced by the University of Canterbury and GenØk – Centre for Biosafety. This is a free-to-the-public resource for hazard identification and risk assessment of genetically modified organisms.

All page numbers not directly referenced refer to the document Part 1 of the technical dossier "Application for authorization to place on the market AV42-6-G7 potato in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed", submitted by the Applicant.

Key findings

After a detailed analysis of many of the portions of the dossier on AV42-6-G7 submitted by the Applicant, we outline a number of informational, methodological and conceptual weaknesses including:

• lack information on potential relevant adverse effects

• improper assumptions;

• lack of information regarding social utility and sustainability aspects

Therefore our input focuses on a critique of the Applicant's dossier and covers three broad issues:

1. Missing, incomplete or inadequate information to support the Applicant's claims

2. Improper assumptions and/or unsupported reasoning by the Applicant related to assessment needs

3. Missing information in relation to requirements under the Norwegian Gene Technology Act

Within we suggest appropriate action to address the specific deficiencies where possible, and conclude our assessment with a summary recommendation.

Lastly, Codex Alimentarius guidelines allow Norway to ask for specific data of the type we identify and recommend obtaining below. Norway therefore may request this information without concern of a challenge from the World Trade Organisation.

These deficiencies seriously undermine any scientifically justified overall conclusion of safety. Key deficiencies include information surround a number of factors:

1. The rates, types and potential pathways of exposure to AV42-6-G7 potato in the Norwegian diet have not been sufficiently characterized by the Applicant. This is essential information to properly characterize risk.

2. Critically, the Applicant has not provided a convincing case for having either identified or analysed off-target effects of the novel dsRNAs or new ones expressed in AV42-6-G7 potato, sufficiently characterized fusion proteins, or other unintended metabolic changes.

3. It is significant that the Applicant has not investigated the production of novel small peptides that mayb be produced by regular but low level expression of intended dsRNAs. The Applicant has only argued that they do not exist, and this argument lacks scientific basis. Thus, the molecular characterization is unsatisfactory for assuring that there are no novel protein-based hazards.

4. Concerning the social utility of AV42-6-G7 potato, outlined in Appendix 4 Part V of the Norwegian Gene Technology Act, it is highly questionable whether this genetically modified variety of amylose-free potato offers and benefit to Norwegians in comparison to the non-GM amylose free potato varieties available or if AV42-6-G7 demonstrates a positive contribution to sustainable development.

Recommendations

Based on our findings, we propose a number of specific recommendations, summarized here and detailed in the critique below.

The Direktoratet for naturforvaltning is encouraged to request:

1. The Applicant should provide empirical information to verify that the probes used would detect smaller or rearranged transgenic fragments that may be integrated into host genome at a limit of detection of ≤ one target/tetraploid genome.

2. The Applicant should provide additional data using a comprehensive set of smaller probes to establish the presence or absence of backbone vector DNA sequences at a limit of detection of ≤ one target/tetraploid genome.

3. Given the deletions reported after integration of the transgenic DNA into the host genome, the Developer should provide a survey of the actual RNAs produced or absent at the integration junctions.

4. The Applicant should provide direct proof of the absence of any residual DNA from the antibiotic resistance marker gene *NptII*.

5. The Applicant should provide direct proof of the absence toxicological or allergenic effects from the creation of fusion proteins following transformation of event AV43-6-G7.

6. The Applicant should supply information on all RNA molecules unique to event AV43-6-G7, or at unique concentrations in event AV-43-6-G7, all off-target changes to gene expression in event AV43-6-G7, and the potential for the novel molecules (or molecules at novel concentrations), and possible derivatives that may be made in human cells, to cause effects on human cells. Moreover, that information should be informed by appropriate high throughput sequencing methodologies.

7. The Applicant should indicate how they will monitor ongoing nucleotide-level changes in the transgene and subsequent changes to the off-target effects of the dsRNA. In the absence of such monitoring, approval should be conditional and limited to a period of no more than three years.

8. As the oral toxicity tests contain numerous design flaws that confound the identification of relevant effects, the applicant should follow up on and report further on the significance in reported gender effects.

9. The DN should request additional information to support the claims of agronomic superiority of AV43-6-G7 over existing non-GM parental lines, and consider this

information in light of the consideration of social utility outlined in the impact assessment provisions under Appendix 4 of the Norwegian Gene Technology Act.

10. Given that the application is for approval for use in food and feed, the Applicant should produce a safety evaluation of the chemical composition of co-products intended for human or animal consumption, including target proteins after processing, and including feeding studies.

11. The Applicant should submit required information on the social utility of AV43-6-G7 potato and its contribution to sustainable development, and further information on cultivation in the Norwegian context, in accordance with the Norwegian Gene Technology Act.

Overall recommendation

Above we highlight a number of conceptual, empirical and informational deficiencies of the dossier to support claims of safe use, social utility and contribution to sustainable development of AV43-6-G7. Critically, the Applicant has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway. Taken together, these deficiencies fail to address the necessary safety regulations under Norwegian Law, and thus the application is incomplete and should not be approved. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of AV43-6-G7 potato we conclude that based on the available data, including the safety data supplied by the Applicant, the Applicant has not substantiated claims of safety satisfactorily to warrant approval in Norway at this time.

Assessment of the technical dossier related to EFSA/GMO/NL/2011/69

About the event

The transgenic potato event AV43-6-G7, developed by "AVEBE" U.A., has been genetically engineered to restrict the production of amylose though the inclusion of an inverted repeat iRNA construct of the Granule Bound Starch Synthase I gene (GBSSI) region from potato. The inverted repeat iRNA construct of GBSSI prevents the expression of the endogenous GBSSI gene and thereby reduces the amount of amylose in starch, facilitating starch extraction and use for industrial purposes.

1. Missing, incomplete or inadequate information to support the Applicants claims

1.1 Molecular characterization of the inserted DNA

1.1.1 Copy number of T-DNA

To determine the copy number of event AV43-6-G7, the Applicant used 4 probes for Southern blotting analysis. These probes ranged in size from 528bp-1.3 kb.

However, the Applicant has not provided sufficient data to determine what minimum size of target is necessary for hybridization with and hence detection with these probes. In other words, the size of the probes was not validated for ability to detect smaller or rearranged fragments with partial overlap at the single stringency used to wash the blots. This should be done to a stated detection limit, preferably ≤ one target/tetraploid genome (BAT).

The probes used could only possibly survey less than half of the T-DNA (3 kb of the 6069bp T-DNA). Only two of the probes (Vsp1 and BINMSC/GBSS1) overlapped. The Applicant should have used a panel of probes that collectively surveyed the entire vector and T-DNA (BAT). This is absolutely necessary to make a reasonable claim that there were no partial and/or rearranged T-DNA sequences integrated elsewhere in the genome.

Taking together the above problems in methodology and reporting, there is insufficient evidence to claim that "Event AV43-6-G7 contains a single, truncated, T-DNA copy of construct pKGBA50mf-IR1.1" (p. 13/95 Wolters and Visser, 2010).

Recommendation: The Applicant should provide empirical information to verify that the probes used would detect smaller or rearranged transgenic fragments that may be integrated into host genome at a limit of detection of ≤ one target/tetraploid genome.

1.1.2 Determination of absence of backbone vector DNA/unintended transgenes in event AV43-6-G7

To detect integration of unintended (backbone) sequences, the Applicant performed a combination of PCR and Southern blotting. This was done because 'backbone' transfers are common when introducing recombinant DNA using the Ti plasmid system found in Agrobacterium. Historical data underestimates the number of backbone transfers because: "Usually, transfer of only the non-T-DNA sequences to the plant would remain undetected because: (1) there is no selection for the transfer of such sequences; and (2) scientists generally have not looked for the transfer of these sequences" (Kononov et al., 1997). The amount of DNA that can transfer can be many times the length of the T-DNA region "extremely long regions of DNA (greater than 200 kbp) can transfer to and integrate into the genome of plants" (Kononov et al., 1997). Short backbone sequences can transfer and be difficult to detect. "In many instances, vector 'backbone' regions of a binary vector are smaller than what is conventionally termed the 'T-DNA' region" (Kononov et al., 1997).

The PCR coverage was at best capable of surveying only 5.4 of the 8 kbp of backbone DNA. Moreover, PCR would have detected only inserts that preserved the expected order of sequences targeted by the primers and only those that were separated by a number of base pairs that could be reasonably amplified. Partial inserts or rearranged sequences that lost the primer sequences would have gone undetected by PCR.

The Applicant also used Southern blotting to raise confidence in the conclusion that there were no insertions of unintended material. Unfortunately, in this case a single 8 kbp probe corresponding to the entire backbone sequence was used. Such large probes are prone to giving false negative results because small inserts would not retain the probe during high stringency washing of the blot (65°C, 0.5-1 x SSC). The Applicant has not justified this stringency and has not validated it for surveying this genome (see above). The Applicant should have used a comprehensive set of much smaller probes (BAT).

Taking together the above problems in methodology and reporting, there is insufficient evidence to claim that "Event AV43-6-G7 does not contain any backbone vector DNA sequences" (p. 27/95 Wolters and Visser, 2010).

Recommendation: The Applicant should provide additional data using a comprehensive set of smaller probes to establish the presence or absence of backbone vector DNA sequences at a limit of detection of ≤ one target/tetraploid genome.

1.1.3 Sequence analysis of event AV-43-6-G7

The Applicant found significant deletions of DNA in the characterized insertion relative to the expected sequence prior to transfer from Agrobacterium. Of note was a 237 bp deletion on the Right Border side and a 1056 bp deletion on the Left Border side (Figure 28 of Wolters and Visser, 2010). The LB side deletion includes the LB sequence, the NOS terminator and part of the GBSS1 antisense fragment.

In addition, 1814 bp of genomic DNA was deleted in the event chromosome. Thus, in addition to new junctions caused by insertions of recombinant DNA and thus possible novel RNAs in the transcriptome and proteins in the proteome (see below), there may be a loss of endogenous RNAs and proteins that have no apparent effect on agronomic qualities but may have an effect on the expression or accumulation of toxins or anti-nutrients. The bioinformatic analysis provided by the Applicant does not substitute for a survey of actual RNAs produced at the junctions or for a survey of deleted RNAs.

Recommendation: Given the deletions reported after integration of the transgenic DNA into the host genome, the Developer should provide a survey of the actual RNAs produced or absent at the integration junctions.

1.1.4 Presence/absence of antibiotic resistance marker gene

"Binary vector pKGBA50 was digested with enzymes PmeI and ClaI to remove the NptII gene" (p. 7/95 of Wolters and Visser, 2010). Technical details in dossier (Wolters and Visser, 2010) or in underlying reference (Kuipers, 1995) do not make clear whether all or some of the DNA sequence originally annotated as nptII was removed. If this was a partial deletion, then risks associated with recombination and reactivation of NptII activity, or with horizontal gene transmission using the residual nptII DNA as an anchor, should be addressed.

Recommendation: The Applicant should provide direct proof of the absence of any residual DNA from the antibiotic resistance marker gene *NptII*.

1.1.5 Open reading frame (ORF) analysis

As part of the molecular characterization of event AV43-6-G7, the Applicant examined the 5' and 3' junctions between the T-DNA and chromosomal sequences for the presence of new ORFs. Two fusion-ORFs that span the junctions were identified and further analyzed by the Applicant. This is in accordance with Codex Alimentarius guidelines:

> §31
> Information […] should include: …
> D) identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins (emphasis added to p. 14 Codex, 2003b).

Further, Codex Alimentarius states that:

> §33
> In addition, information should be provided: …
> E) to indicate whether there is any evidence to suggest that one or several genes in the host plant has been affected by the transformation process; and
> F) to confirm the identity and expression pattern of any new fusion proteins (p. 15 Codex, 2003b).

Using a bioinformatics approach, the Applicant found that the N-terminal part of the putative fusion protein Orf4 (152 aa) shows high similarity with an EST (GenBank: CN212569.1) derived from *Solanum tuberosum*, which means it can be expressed in the host. The C-terminal part of Orf4 consists of an additional 24 amino acid bases, derived from the *lacI* repressor present on the T-DNA. The Applicant concludes that as

a) a blastp search did not reveal a significant similarity, and
b) "[…] the *lacI* repressor protein is not a known allergenic or toxic protein, and the 24 amino acids of *lacI* present in ORF1 are not part of a functional domain, the ORF4 peptide is not expected to be toxic or allergenic" (p. 71 of Wolters, 2010).

Orf1is in the same region but in a different reading frame than orf4, and "[a] low level of similarity was observed with a putative integron gene cassette protein" (p. 72 of Wolters, 2010).

To conclude that a novel protein is likely to be of no safety concern because of the addition of 24 amino acids from a known protein is not a research-based conclusion. For example, the change of only two amino acids in the gm-hra gene used in soybean DP-305423-1 is enough to confer tolerance to ALS-inhibiting herbicides. Changes of single amino acids can drastically alter the characteristics of proteins (e.g. Doyle and Amasino, 2009, Hanzawa et al., 2005, Zubieta et al., 2008), a fact that underpins the field of directed evolution (reviewed in e.g. Bloom and Arnold, 2009, Tracewell and Arnold, 2009). One of the characteristics that can be changed is immunogenicity. For example, several groups reported significant decreases of IgE binding to a major peanut allergen after mutating single nucleotides (Glaspole et al., 2005, King et al., 2005, Ramos et al., 2009). Even more surprising, in some cases not even an amino acid change is necessary to alter the characteristics of a protein. Kimchi-Sarfaty et al. demonstrated that even synonymous single nucleotide polymorphisms (i.e. differences in the nucleotide sequence of a gene that do not alter the resulting amino acid sequence) can change the substrate specificity of the resulting protein, potentially by affecting its folding patterns during translation (Kimchi-Sarfaty et al., 2007). Changes in the tertiary structure alone can turn benign proteins into toxins (Bucciantini et al., 2002, Ellis and Pinheiro, 2002, Ross and Poirier, 2005), as demonstrated for the Prp proteins causing Creutzfeld-Jacob disease and mad cow disease (Caughey and Baron, 2006). 24 new amino acids are therefore more than enough to cause biological effects. It is only through proper scientific testing that unintended or unanticipated effects caused by the new ORF can be ruled out.

Recommendation: The Applicant should provide direct proof of the absence toxicological or allergenic effects from the creation of fusion proteins following transformation of event AV43-6-G7.

1.1.6 Transcriptome analysis of the dsRNA modification(s)

The modification of AV43-6-G7 is based on dsRNA silencing, which has not benefitted from human food safety studies to our knowledge. There are sufficient reasons to require a higher level of analysis for dsRNA modifications because they are based on still developing science,

and thus dsRNA should not be generally regarded as safe (GRAS). A key concern is that the full transcriptome of the product has not been carefully evaluated for small RNAs or small peptides with toxic and or immunomodulatory properties.

Research by the Monsanto Corporation has shown that novel dsRNA molecules at unique concentrations in transgenic plants can transfer through food to animals wherein these molecules or derivatives of these molecules cause adverse effects (Baum et al., 2007). Researchers demonstrated that dsRNA can be infectiously transferred through food to gut cells in insects, and subsequently spread within the animals (Gordon and Waterhouse, 2007). The dsRNA created in the transgenic dsRNA-insecticide plants were in fact derivative or "secondary" RNA species, and notably Baum et al. (2007) are sure that they were the cause of more derivative RNA molecules after processing by the RNAi activity in the target insects (that is, not present in this form in the plants). The Applicant should have conducted both food safety and environmental safety assessments to demonstrate that secondary processing in human cells, or in the gut of important indicator species, of novel dsRNA molecules created by event AV-43-6-G7 would not generate a biologically active dsRNA.

A history of consuming small RNA molecules in plants is not the same as extrapolating the safety of all small RNA molecules, any more than a history of consuming proteins attests to the safety of every protein. When a small RNA molecule will or might not act as a gene regulator is not always known in advance. Therefore, it cannot be assumed that novel small RNAs that might be created in event AV43-6-G7 will likewise be safe. Certainly, dsRNA used as an insecticide is not safe from the perspective of pest insects targeted in other work described above (Auer and Frederick, 2009, Baum et al., 2007) and by extrapolation some small RNAs may not be safe for humans. Indeed, the plants that humans traditionally consume may be precisely those that produce small RNAs that have not been toxic to us.

It is now clear that dsRNA can have significant biological impact. Recent research (Baum et al., 2007, Gordon and Waterhouse, 2007, Mao et al., 2007) establishes beyond doubt that novel RNAs of recombinant or synthetic origin cannot be GRAS but must be tested and demonstrated to be safe. The insecticide findings provide powerful argument for proper profiling of the transcriptome and proteome in human health and environment safety assessments of GM crops to now accept the importance of such enquiry (Heinemann, 2009).

Moreover, dsRNA molecules generate many off-target effects that may significantly alter the range and concentration of normal metabolites (BAT, Heinemann, 2009). Unless the Applicant has conducted a complete profile of the transcriptome, additional off-target effects could be missed.

The genes silenced by dsRNAs are specific to the dsRNA, rather than dsRNAs are specific to target genes (Jackson et al., 2003). Sometimes hundreds of off-target transcripts are reduced or silenced (Jackson et al., 2003, Jackson et al., 2006, Jackson and Linsley, 2004, Ma et al., 2006). For example, Semizarow et al. found that a set of 5 different dsRNA molecules that silence the same gene (AKT1) collectively silenced 840 genes (Semizarov et al., 2003). Species-specific differences in RNA editing further contribute to unanticipated dsRNA species and off-target effects (O'Connell and Keegan, 2006). Therefore, the transcriptome of event AV43-6-G7 should be evaluated for all novel dsRNAs. Second, off-target effects

sometimes only change protein levels and not transcript levels (Jackson and Linsley, 2004, Scacheri et al., 2004), making it even more complicated to track effects. Therefore, both the transcriptome and proteome of the GM crop should be profiled.

"[F]urther research into off-target effects should be encouraged because the current lack of information creates uncertainties about this particular hazard" (p. 6 of 8 Auer and Frederick, 2009).

High-throughput sequencing has proven to be a powerful and quantitative method to sample transcriptomes deeply at maximal resolution. In contrast to hybridization, sequencing showed little, if any, background noise and was sensitive enough to detect widespread transcription in >90% of the genome, including traces of RNAs that were not robustly transcribed or [were] rapidly degraded (p. 1239 Wilhelm et al., 2008).

Additionally, researchers have applied this technique to organisms at different stages of their life cycles and under different environmental conditions, demonstrating that this technique can be effectively used to describe the transcriptome of different tissues, stages of development and at different times (Wilhelm et al., 2008). It can be used on any kind of GMO (Lu et al., 2007).

Not only has full transcriptome profiling become possible, it is also seen as "necessary to sample the full complexity of small RNAs in plants and likely other organisms as well. Application of this method to several key mutants affecting small RNA biogenesis pathways can quickly lead to the identification of candidate miRNAs, trans-acting siRNAs and other interesting classes of small RNAs" (p. 116 Lu et al., 2007). The sequencing technique is less prone than global microarrays to ambiguities due to background detections (Kristensen et al., 2005, Wilhelm et al., 2008).

Codex Alimentarius allows countries to ask for information on RNA molecules without concern of action from the WTO:

"Information should be provided on any expressed substances in the recombinant-DNA plant [or microorganism]; this should include: A) the gene product(s) (e.g. a protein or an untranslated RNA)…E) where possible, the amount of the target gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the accumulation of a specific endogenous mRNA or protein" (p. 14 and 39 of Codex, 2003a).

We recommend that information be requested from the Applicant on all RNA molecules unique to event AV43-6-G7, or at unique concentrations in event AV-43-6-G7, all off-target changes to gene expression in event AV43-6-G7, and the potential for the novel molecules (or molecules at novel concentrations), and possible derivatives that may be made in human cells, to cause effects on human cells. Moreover, that information should be informed by appropriate high throughput sequencing methodologies.

Finally, there is evidence that "[m]utation rates in genes for small RNAs can be high relative to protein-coding genes" (p. 5 of 8 of Auer and Frederick, 2009). Thus, approval of GMOs that rely on small RNA molecules for their effects may not be suitable for a single approval

regulatory system because changes in these sequences over time can lead to further and unanticipated off-target effects.

Recommendation: The Applicant should supply information on all RNA molecules unique to event AV43-6-G7, or at unique concentrations in event AV-43-6-G7, all off-target changes to gene expression in event AV43-6-G7, and the potential for the novel molecules (or molecules at novel concentrations), and possible derivatives that may be made in human cells, to cause effects on human cells. Moreover, that information should be informed by appropriate high throughput sequencing methodologies.

Recommendation: The Applicant should indicate how it will monitor ongoing nucleotide-level changes in the transgene and subsequent changes to the off-target effects of the dsRNA. In the absence of such monitoring, approval should be conditional and limited to a period of no more than three years.

1.2 Oral toxicity studies

There are a number of factors in the oral toxicity test that make it difficult to draw conclusions about the safety of AV43-6-G7. For instance, it is unclear why the applicant added variable amounts of potato starch to all diets, including the control diet 1. Diet 1 is given 60.42 % potato starch, Diet 2-33.17%, Diet 3-46.82 % and Diet 4-33.22 %. The applicant claims that the availability of nutrients in raw potato are the same as in the added potato starch, but no evidence is given to justify this claim. This is an important point because if this is not the case, then the high dose group would in fact be given less nutrients than the low dose group and the control group.

There were significant differences in body weight and water consumption, but the relevance are difficult to assess because the applicant do not adequately described them. Instead they describe the occurrence as “occasionally” and “at a few stages, they (i.e. body weight) were also statistically significantly increased….”

As mentioned above, there are a number of statistically significant differences between the control group and the treatment groups. The applicant claims that this is due to the effect of raw potato added to the diet because it also occur in the reference control group (WT-Potato). Although this is a necessary test for possible environmental effects (e.g. relevant for wildlife eating potatoes in the field), raw potatoes will not be administered to animals and certainly not to humans as raw unprocessed material added to the diet. The applicant should in addition test the actual product being used in animal feed/human food (cooked and/or heat treated).

An overview over the statistically significant differences between the groups are given in Appendix 6 – page 26. The applicant has in the oral toxicity test discovered numerous statistically significant differences between the test groups that they argue are not relevant because the effect occurs in one sex only, or that the effect do not have a clear dose-response relationship. We believe that this conclusion is incorrect. Two recent articles from Seralini and coworkers (2009, 2011) have discussed gender related and non-linear characters of

toxicity, where they refer to examples of well documented gender- and endocrine disruptive effects that do not necessarily have such a linear relationship (often J or U shaped curves). Such effects could arise at certain time points depending on the age and exposure of the test individuals. Therefore we believe that significant differences between test groups cannot be discounted on the basis of that they do not occur in both sexes or that they do not have a linear relationship between dose and response (Seralini et al., 2009; Seralini et al., 2011).

Recommendation: As the oral toxicity tests contain numerous design flaws that confound the identification of relevant effects, the applicant should follow up on and report further on the significance in reported gender effects.

2. Improper assumptions and/or unsupported reasoning by the Applicant related to assessment needs

2.1 Agronomic performance of the Applicant's genetically modified (GM) vs. available non-GM amylose-free potatoe varieties

Early in the dossier, the Applicant rationalizes this GM version of amylose-free potato over exisiting non-GM versions as providing a benefit to disease resistance and increase phytosanitary compliance in some districts in the Netherlands and Germany:

"This application comprises a genetically modified amylopectin starch potato AV43-6-G7 with good agronomical [sic] characteristics. The amylopectin trait in potato is not new to the (Food and Feed) market. Current non-GM commercial available amylopectin potato varieties lack important disease resistances and can only be grown in specific regions. For the main starch potato area of AVEBE in The Netherlands and adjacent German region these non-GM varieties are not allowed to be grown because of phytosanitary regulations." (p.10)

Yet the Applicant gives no empirical evidence of the claimed increase in resistance. Further, the hyperlinks to qualitative differences between the parent Karnico potato and the modified version AV43-6-G7 were not functional (attempted Feb 22, 2011 and March 2, 2011).

Despite this claim, the Applicant's actual experimental data on agronomic performance between the partental line and AV43-6-G7 found no significant difference in resistance to agronomically important potato diseases:

"With regard to resistance against a number of diseases (late blight, virus, potato cyst nematodes and wart disease) no differences were found between AV43-6-G7 and Karnico except for a minor deviation in virus resistance score. We conclude that the biology of the plant is unchanged with respect to these parameters. This makes AV43-6-G7 in agronomic terms superior to the non-GM amylopectin varieties which are presently already grown commercially." (p46)

Thus, the Applicant' own data does not support that "AV43-6-G7 in agronomic terms superior to the non-GM amylopectin varieties which are presently already grown

commercially." (p46). This calls seriously into question the utility of this GM variety over available non-GM varieties that do the same thing (discussed further in section 3.1)

Recommendation: The DN should request additional information to support the claims of agronomic superiority of AV43-6-G7 over existing non-GM parental lines, and consider this information in light of the consideration of social utility outlined in the impact assessment provisions under Appendix 4 of the Norwegian Gene Technology Act.

2.2 Effect of processing

With respect to possible changes incurred by processing (which may include heating, chemical extraction, etc), the Applicant assumes:

Compositional variation due to changes in today's processing may have nutritional (and economical) implications for the co products, however, are not a safety issue. AV43-6-G7 is compositionally equivalent to the tubers from its conventional counterparts. A separate risk assessment on the processed products is therefore deemed not to give any additional information and for AV43-6-G7 risk analysis based on the whole tuber is adequate." (p.47).

Yet we find no empirical reason why this assumption of safety is valid. Given that the Applicant has applied for AV43-6-G7 to be valid for use in food and feed, the Applicant should supply information on the compositional changes that would arise from treatment and further use of co-products (e.g. potato pulp) in livestock and possibly human consumption.

Recommendation: Given that the application is for approval for use in food and feed, the Applicant should produce a safety evaluation of the chemical composition of co-products intended for human or animal consumption, including target proteins after processing, and including feeding studies.

3. Missing information in relation to requirements under the Norwegian Gene Technology Act

3.1. 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. In accordance with the aim of the Norwegian Gene Technology Act, 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 detailed in the regulation on consequence assessment section 17 and its annex 4. The Applicant has not provided relevant information that allows an evaluation of the issues laid down in the aim of the Act, regarding ethical values, social justification of the GMO within a sustainable development. 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 AV43-6-G7. The Applicant should thereby provide the necessary data in order to conduct a thorough assessment on these issues, or the application should be refused.

In comparison with many earlier GMO applications, it is important to emphasis that potatoes is grown extensively in Norway and has both a traditional and cultural value for the Norwegian people. It is also important to evaluate whether alternative options, (e.g. the parental non-GM version of this potato) has achieved the same outcomes in a safer and ethically justified way.

Further, 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. For instance, it is difficult to extrapolate on hazards or risks taken from data generated under different ecological, biological, and genetic 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. Hence it cannot be expected that the same effects will apply between different environments and across continents.

Approval of a GMO in Norway is dependent on that the GMO in question has been thoroughly tested in the environments in which the GMO can be released (section 15, regulation on Consequence assessment under the Gene Technology Act). In other words, because of the differences in agroecosystems noted above, the potato in question has to be thoroughly tested under Norwegian conditions before an application can be approved. The Applicant has not provided such information.

Recommendation: The Applicant should submit required information on the social utility of AV43-6-G7 potato and its contribution to sustainable development, and further information on cultivation in the Norwegian context, in accordance with the Norwegian Gene Technology Act.

Conclusion

Available information for risk assessment evaluation

This evaluation is for the most part based on the Applicant's own submitted information. The directly relevant scientific literature is very limited in some cases, yet 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.

The lack of compelling scientific information to support the claims of the Applicant highlights the need for independent evaluation of safety studies and molecular information. We therefore request that mechanisms become available that allow to all information, including annexes that explain confidentiality claims invoked for some of the application information that may be of scientific relevance. Such independent evaluation is essential to maintaining rigorous standards expected in scientific practice. In this particular case, the Applicant has not requested confidentiality normally experienced in other applications. Despite the deficiencies in the dossier under examination here, we encourage the authorities to insist on this level of transparency and accessibility to raw data the Applicant has given to apply to all future dossiers to be considered.

Overall recommendation

Above we highlight a number of conceptual, empirical and informational deficiencies in the dossier that do not justify a conclusion of safe use, social utility and contribution to sustainable development of AV43-6-G7. Critically, the Applicant has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway. Taken together, these deficiencies fail to address the necessary safety regulations under Norwegian Law, and thus the application is incomplete and should not be approved. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of AV43-6-G7 potato we conclude that based on the available data, including the safety data supplied, the Applicant has not substantiated claims of safety satisfactorily to warrant approval in Norway at this time.

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