



GenØk - Centre for Biosafety

Vår ref:2011/h88  
Deres ref: 2011/5292 ART-BI-BRH

Direktoratet for naturforvaltning  
Tungasletta 2  
7485 Trondheim

Dato: 14.06.2011

Kjare Bjarte,

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet om høring 88 for genmodifisert potet AM04-1020 fra BASF Plant Science Company GmbH for import, prosessering, dyrking, og bruk i mat og fôr.

Hvis du har noe spørsmål, vennligst ta kontakt.

Med vennlig hilsen,

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**Assessment of the technical dossier submitted under EFSA/GMO/SE/2010/88  
for approval of transgenic potato event AM04-1020 by BASF Plant Science  
Company GmbH**

**Submitted to**  
**Direktoratet for Naturforvaltning**

**by**  
**Centre for Biosafety – GenØk**  
**and**  
**Center for Integrated Research in Biosafety**  
**June 2011**

**Innspill til offentlig høring av søknad EFSA/GMO/SE/2010/88****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 AM04-1020 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 brukt i norsk mat 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, som medfører at risiko ikke kan vurderes på en god måte.
2. Søker har ikke lagt ved en adekvat molekylær karakterisering av det innsatte konstruktet, og har heller ikke gjennomført en tilfredstillende analyse med hensikt å demonstrere at integrering av konstruktet ikke har ført til utilsiktet effekt på endogen gen funksjon.
3. 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 AM04-1020 potet. De har ikke karakterisert fusjonsproteiner tilstrekkelig. Det samme gjelder for undersøkelse av eventuelle metabolske forandringer.
4. Toksisitet testen som er utført inneholder flere muligheter og antagelser i studie design som påvirker identifikasjon av mulige uønskede skadelige effekter. Mulige eksponeringsveier er ikke tilstrekkelig karakterisert til å klargjøre risiko.
5. Søker har ikke undersøkt eventuell produksjon av nye små peptider forårsaket av lavnivå uttrykk av dsRNA. Søker har kun argumentert for at disse ikke eksisterer, men dette argumentet mangler vitenskapelige bevis. Den molekylære karakteriseringen er dermed for utilstrekkelig til å kunne gi en konklusjon om at det ikke dannes nye unike protein-baserte farer.
6. Analyse av søker viser at det er statistisk signifikante forskjeller i nitrat nivåene hos

den GM poteten. Til tross for dette vurderer søker at disse forskjellene er normale basert på sammeligning med referanse linjer av potet som har vært dyrket på på andre steder eller tidligere år. En slik bruk av referanselinje for vurdering av nærings sammensetning er ikke i overensstemmelse med nåværende veiledning for bruk av referansepunkt/ komparatorer.

7. Søker har ikke gitt tilfredstillende informasjon om hvordan planer for overvåking skal utføres. Vår vurdering av søkers foreslåtte overvåkningsplaner tilfredstiller ikke overvåkings krav i henhold til Directive 2001/18/EC og Council Decision 2001/811/EC.
8. Når man ser på den Norske genteknologiloven, appendix 4 del V, er det høyst tvilsomt om AM04-1020 oppfyller de krav som stilles i loven om samfunnsnytte. Etter vårt skjønn e representerer bruk av den amylose-reduerte genmodifiserte poteten ingen fordel for hverken norske forbrukere, bønder eller produsenter i og med at det allerede finnes ikke-GM amylose reduserte potetvarianter på markedet. Det er også høyst tvilsomt om AM04-1020 er et positivt bidrag til bærekraftig utvikling.

### Hovedkonklusjon og anbefalinger

Vi har i vår gjennomgang av vedlagt dokumentasjon 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 AM04-1020. 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. Videre anbefaler vi også at en eventuell ny søknad kun bør vurderes om søker har adressert de mangler vi har belyst.

## Summary of the assessment of the technical dossier related to EFSA/GMO/SE/2010/88

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 AM04-1020, 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 of Amylopectin Potato BPS-A1Ø2Ø-5 for Food and Feed Uses, Processing and Cultivation according to Regulation (EC) No 1829/2003”, submitted by the Applicant.

### Key findings

After a detailed analysis of many of the portions of the dossier on AM04-1020 submitted by the Applicant, we outline a number of informational, methodological and conceptual shortcomings, based on the given data, that do not justify the Applicant’s conclusion of safety.

Our input focuses on a critique of the Applicant’s dossier and covers three broad issues:

1. Flawed assumptions, reasoning, or interpretations by the Applicant in the use of its data
2. Missing, incomplete or inadequate information to support scientifically sound claims of safety
3. Missing information in relation to requirements under the Norwegian Gene Technology Act

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

The highlighted deficiencies seriously undermine any scientifically justified overall conclusion of safety.

**Key informational deficiencies surround a number of factors:**

1. The rates, types and potential pathways of exposure to AM04-1020 potato in the Norwegian diet have not been sufficiently characterized by the Applicant. This is essential information to properly characterize risk.
2. The Applicant has not produced an adequate molecular characterization of the putative insert, nor sufficiently conducted the analysis to demonstrate that the integration event has not disrupted endogenous gene function.
3. 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 AM04-1020 potato, sufficiently characterized possible read-through expression, leading to fusion proteins, or other unintended metabolic changes.
4. The oral toxicity tests contain numerous choices and assumptions in the design of the studies that confound the identification of relevant adverse effects. Exposure pathways are not sufficiently characterized to infer possible risk.
5. It is significant that the Applicant has not investigated the production of novel small peptides that may be produced by regular but low level expression of intended dsRNAs.
6. The compositional assessment identified statistically significant differences in nitrate levels. Yet the Applicant considered these differences normal on the basis of extended comparison with reference lines of potato grown on at other locations or years, which does not conform to current guidance on the use of comparators.
7. The Applicant has not given sufficient details on how the monitoring plans will meet the stated objectives. Based on our analysis the proposed monitoring plans does provide sufficient detail to ensure that the main requirements of monitoring outlined in requirements Directive 2001/18/EC and Council Decision 2001/811/EC will be fulfilled.

8. Concerning the social utility of AM04-1020 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 AM04-1020 demonstrates a positive contribution to sustainable development.

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.

## 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  $\leq$  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  $\leq$  one target/tetraploid genome.
3. Given the deletions reported after integration of the transgenic DNA into the host genome, the Applicant should provide a survey of the actual RNAs produced or absent at the integration junctions and in the DNA surrounding the insert, preferably using high throughput transcriptome sequencing techniques.
4. The Applicant should determine experimentally if the T-DNA insertion disrupted expressed sequences in event AM04-1020.
5. The Applicant should provide experimental evidence that no rearrangements, deletions or insertions occurred around the insertion site of event AM04-1020, or that any detected rearrangements, deletions or insertions do not lead to any adverse effects.
6. The Applicant should supply information on all RNA molecules unique to event AM04-1020, or at unique concentrations in event AM04-1020, all off-target changes to gene expression in event AM04-1020, 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 oral toxicity tests contain numerous choices and assumptions (described above) in the design of the studies that confound the identification of relevant effects. Exposure pathways are not sufficiently characterized to infer possible risk. The Applicant to perform oral toxicity studies that conform to minimum guidelines set by EFSA.
8. The Applicant should be required to further investigate the apparent increased propensity for AM04-1020 to accumulate nitrate in tubers compared to conventional varieties.
9. The Applicant should be required to repeat nitrate quantification measurements on tuber samples using a greater sensitivity and thus, a lower limit of quantification. The Applicant should also restrict comparisons to the isogenic comparator only and not include data for reference lines grown under different conditions, times, or localities.
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 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.
12. The Applicant should provide a monitoring plan that tests the assertions of safety made within the environmental risk assessment.
13. The Applicant should be required to revise and resubmit the application with a monitoring plan the more specific details on monitoring objectives, e.g. monitoring design, areas to be monitored, which monitoring networks will be engaged, or how the data will be analyzed.
14. The Applicant should submit required information on the social utility of AM04-1020 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**

Below 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 AM04-1020. 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. Further the monitoring proposed by the Applicant does not contain sufficient details to ensure the key provisions of monitoring under the or Appendix III of the 2005 revised regulations of the Norwegian Gene Technology Act or of Directive 2001/18/EC and Council Decision 2001/811/EC. 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 as is. 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 AM04-1020 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.

## Assessment of the technical dossier related to EFSA/GMO/SE/2010/88

### About the event

The transgenic potato event AM04-1020, developed by BASF Plant Science Company GmbH has been genetically engineered to restrict the production of amylose through the inclusion of an inverted repeat iRNA construct of the Granule Bound Starch Synthase (GBSS EC 2.4.1.242) originating from *Solanum tuberosum*. The inverted repeat iRNA construct of GBSS prevents the expression of the endogenous GBSS 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 of event AM04-1020

To determine the copy number of T-DNA inserts, the Applicant used 3 probes for Southern blot analysis: gbss (ca 1700 bp), nos (ca 900 bp), csr1-2 (ca 2000 bp). Positive controls (DNA from the mother variety spiked with 1 and 2 genome equivalents of plasmid pAP4) showed that the probes were “sensitive enough to detect a single-copy T-DNA insert” (p. 216, Annex 2). However, the Applicant has not provided sufficient data to determine the minimum size of the target or what structure it needs to display to allow 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 and/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  $\leq$  one target/tetraploid genome ([www.bat.genok.org/bat](http://www.bat.genok.org/bat)). Additionally, the probes were not overlapping, and therefore would potentially miss part of the T-DNA had it inserted separately from the full length characterized insert. Taking together the above problems in methodology and reporting, there is insufficient evidence to claim that “... the T-DNA fragment derived from plasmid pAP4 was integrated at a single locus and as one single copy into the potato genome” (p. 37 of the technical dossier).

**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  $\leq$  one target/tetraploid genome.

### 1.1.2 Detection of absence of backbone vector DNA/unintended transgenes in event AM04-1020

'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 Applicant used Southern blotting to raise confidence in the conclusion that there were no insertions of unintended material. Unfortunately, in this case only two probes (ca. 3000 bp and ca. 2900 bp) corresponding to the entire backbone sequence were 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, 0.5-2 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 ([www.bat.genok.org/bat](http://www.bat.genok.org/bat)).

Taking together the above problems in methodology and reporting, there is insufficient evidence to claim that "no elements derived from the backbone of the plasmid pAP4 either linked or unlinked to the insert were detected in the genome of AM04-1020" (p. 37 technical dossier).

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  $\leq$  one target/tetraploid genome.

### 1.1.3. Sequence analysis of event AM04-1020

#### *a) Sequence analysis of the T-DNA in event AM04-1020*

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 150 bp deletion on the Right Border side and a 253 bp deletion on the Left Border side. The LB side deletion includes the LB sequence and part of the NOS terminator (page 19 of Annex 2), which is responsible for proper termination of transcription to reliably produce only the intended transcript, in this case for AHAS. AHAS confers resistance to imidazolinone herbicides and is used as a selectable marker.

Even when intact, the nos terminator is known to create read-through transcripts (Rang et al., 2005). This has been shown to lead to RNA variants that can be further processed, which can produce additional fusion proteins (Rang et al., 2005, Rosati et al., 2008). Recently,

Nicholson and Srivastava (2009) showed that “aberrantly terminated sense transcripts serve as efficient inducers of gene silencing” (p. 319). This was not restricted to the target gene but affected other endogenous genes as well (also see section 1.1.4 below).

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, 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, especially if part of the nos terminator is missing.

Recommendation: Given the deletions reported after integration of the transgenic DNA into the host genome, the Applicant should provide a survey of the actual RNAs produced or absent at the integration junctions and in the DNA surrounding the insert, preferably using high throughput transcriptome sequencing techniques (Heinemann et al., 2011).

*b) Sequence analysis of the chromosomal DNA surrounding the T-DNA in event AM04-1020*

The Applicant sequenced just under 1.1 kb of genomic DNA on either side of the T-DNA insert. The analysis was undertaken to establish “if the insertion disrupts any known CDS or regulatory region” (p. 258, Annex 3). The sequences obtained were compared to different databases: two incomplete and not fully annotated potato genomes, the potato PlantGDB-assembled unique transcripts (PUTs) database (which consist of assemblies of all publicly available ESTs and cDNAs into unique sequences), an in-house assembly of all publicly available ESTs, and NCBI’s nr DNA and nr protein databases.

The main limitation of this approach was that the sequences in the databases were not obtained from the parental variety Kuras, but from various more or less related potato varieties. Sequence differences between event AM04-1020 and the databases can therefore be expected. However, this does not mean that the detected differences are a priori irrelevant to the safety assessment.

Nevertheless, the BLAST searches did not come up empty. Several ESTs and cDNA sequences in the databases showed high similarities to the AM04-1020 DNA1, with one EST (GenBank Accession number CK861784.1) showing 85% identity over 612 bp, starting with the first nucleotide of the potato DNA adjacent to the insert. The sequence of this mRNA, which is coded for on the opposite strand, continues for another 125 nt. This result indicates that a gene might have been disrupted by the T-DNA insertion.

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<sup>1</sup> Right border sequence: potato PUT database PUT40815: 91% identity over 182 bp; in-house database: 4 ESTs with 81% identity over 338 bp, nr DNA database: high similarity over 984 bp

Left border sequence: potato PUT database PUT66865: 95% identity over 406 bp, in-house database: 4 ESTs with 95% over 414 bp; 89% over 501 bp; 89% over 491 bp; 85% over 612 bp)

Recommendation: The Applicant should determine experimentally if the T-DNA insertion disrupted expressed sequences in event AM04-1020.

In addition to the direct disruption of genes or regulatory sequences at the insertion site, *Agrobacterium*-mediated transformation frequently leads to deletions and rearrangements in chromosomal sequences around the insertion site (Latham et al., 2006 and references therein). Zolla et al (2008) conclude that:

[I]t is also evident that the insertion of a single gene does not result in a unique newly expressed protein, but rather in many differently expressed genes with respect to the control. This could be due to the fact that, when the transgene enters the nucleus, many genetic loci are randomly affected by the insertion procedure. (p. 1854 Zolla et al., 2008).

Again, the lack of sequencing data from the parental variety Kuras around the insertion site makes it impossible to determine if endogenous sequences were deleted or rearranged, or if ‘filler DNA’ was introduced during the insertion event (Chen et al., 2003).

Recommendation: The Applicant should provide experimental evidence that no rearrangements, deletions or insertions occurred around the insertion site of event AM04-1020, or that any detected rearrangements, deletions or insertions do not lead to any adverse effects (Heinemann et al., 2011).

In summary, the knowledge of the potato genome is so limited that, *by its own admission*, the data available to the Applicant “*are not suitable to determine the chromosome location of the AM04-1020 potato T-DNA*” (p. 12 of Annex 3). And indeed the only firm conclusions seem to be that “[t]he results indicate that the DNA surrounding the AM04-1020 T-DNA at the insertion site is indeed potato genomic DNA.” (p. 40 of the Technical Dossier) and that “no 100% matching EST or CDS has been identified [...], and none of the homologous regions identified [...] were annotated as regulatory regions” (p. 15 of Annex 3). Given how little is known about all sequences that might have regulatory functions in potatoes and the lack of sequence information of the parental variety Kuras, it is difficult to see how the Applicant can rule out that (a) genes or regulatory sequences were disrupted by the insertion of T-DNA and (b) no changes in expression levels of endogenous sequences occurred.

#### 1.1.4 Transcriptome analysis of the dsRNA modification(s)

The modification of AM04-1020 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.*

There are scientifically justifiable reasons for such an analysis. 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 AM04-1020 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 AM04-1020 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, Semizarov 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 AM04-1020 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 AM04-1020, or at unique concentrations in event AM04-1020, all off-target changes to gene expression in event AM04-1020, 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 (Heinemann et al., 2011).

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 AM04-1020, or at unique concentrations in event AM04-1020, all off-target changes to gene expression in event AM04-1020, 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.

## 1.2 Oral toxicity/nutritional feeding studies

Animal feeding studies were conducted on rats and broiler chickens to test the toxicological and nutritional parameters of amylopectin potato AM04-1020. Issues arising from these analyses are as follows:

- (1) Low percentage of test food in the diet of the rats in the toxicology study.
- (2) Lack of justification for the use of cooked or uncooked potato in the different studies.
- (3) While expected uses are enumerated, the amount of expected intake is not.
- (4) Choice of test animals is limited.

(1) A 90-day oral toxicity study was conducted with rats using raw, whole AM04-1020 potatoes. The rats were fed a maximum dose of 50,000 ppm, comprising 5% of the animals' food. The study states that larger quantities could not be used because of potential differences in caloric and nutrient densities between test substance and controls.

The requisite compositional analysis should have provided the Applicant with information necessary to balance the diets at least between the test substance and isogenic control, increasing the potential test substance in the diet. It is acceptable to artificially formulate a control diet with a similar nutrient profile to the GM variety (EFSA 2011a). Furthermore, these concerns did not stop the Applicant from using 20% GM potato in the broiler chicken feeding study. EFSA guidelines (2011a) state the maximum possible dose should be used in toxicology studies.

(2) The processing of the test substance differed between the feeding studies, using either raw or cooked freeze dried meal. The stated uses of AM04-1020 indicate that it could be consumed in either form. Cooking changes the composition of potato (Augustin et al 1978; Rouch and Tozer, 2004), and justification for the use of either form but not both in the feeding studies is requested.

(3) According to EFSA, "The applicant should provide information on known or anticipated human/animal intake considering all possible routes of exposure" (EFSA, 2011a). Though likely end uses of AM04-1020 are given, the likely quantity of exposure is not. This information will help determine the relevance of both feeding studies, and should include exposure of sub populations such as gluten-intolerant people, who may consumer larger quantities of potato starch in their diet.

(4) The choice of test subjects potentially limited the biological relevance of the feeding studies. For the toxicological studies, it is recommended that at least two mammalian species are used, one of which is not a rodent (EFSA, 2008). Only rats were tested here. It is also recommended that animals under stress, such as raising young, or growing quickly, are also analyzed (EFSA, 2008). This amplifies possible effects of the test substance, and increases the study's relevance to human-length life spans.

For nutritional studies utilizing hens, EFSA recommends including chickens during the laying cycle in the analysis (EFSA, 2011a), which was not done here. There are also concerns with the use of chickens at all for such work, as they have different nutritional needs to humans. A 2004 analysis of chicken feeding studies found that most were unable to detect low to moderate level health effects, and may be insufficient to expose long term effects possible over the human lifespan (Roush and Tozer, 2004).

Recommendation: The oral toxicity tests contain numerous choices and assumptions (described above) in the design of the studies that confound the identification of relevant effects. Exposure pathways are not sufficiently characterized to infer possible risk. The Applicant to perform oral toxicity studies that conform to minimum guidelines set by EFSA.

### 1.3 Compositional analysis

Only one value determined in the compositional analysis of event AM04-1020 fell outside the 95% confidence intervals for the isogenic comparator as well as conventional varieties that were used as additional controls. This was an increase in the nitrate content in tubers of AM04-1020, discussed further below.

The Applicant states that "[e]levated mean levels of nitrate were determined for AM04-1020 as compared to Kuras (184.5 mg/kg fresh weight vs. 147 mg/kg fresh weight)" (p 77, Technical Dossier). This elevated level was calculated to be "outside of the prediction interval (greater than the upper limit)" (p 77 Technical Dossier). Despite the finding that this is a statistically significant difference, the applicant concludes that "[t]he values determined for nitrate in AM04-1020 potato ... might need to be disregarded" (p 77, Technical Dossier), citing issues in measuring nitrate levels in some samples as confounding the calculation. A statistically significant elevation in nitrate levels such as this may have important biological consequences. Nitrate consumption has been associated with methaemoglobinaemia in infants and adults (Greer et al, 2005; EFSA, 2008), increased risk of gastrointestinal cancer (Chang et al, 2010; Oñate-Ocaña et al, 2009) and thyroid disease (Weinhold, 2010).

The World Health Organisation recognises the risks associated with increased dietary nitrate intake and, as such, prescribes the acceptable daily intake of nitrate from all sources at 0-3.7mg/kg body weight per day (WHO, 2002). The Applicant notes that "[n]itrate is not produced in tubers, but it accumulates in the tuber upon uptake from the soil" (p 77, Technical Dossier), however this is no reason to dismiss elevated nitrate levels in AM04-1020. If AM04-1020 has a greater propensity to accumulate nitrate in tuber tissue than does its conventional counterpart, further assessment of the safety of this variety would be required.

Recommendation: The Applicant should be required to further investigate the apparent increased propensity for AM04-1020 to accumulate nitrate in tubers compared to conventional varieties.

The elevated nitrate concentration in AM04-1020 tubers was dismissed due to the use of mean nitrate concentration data that was calculated using estimated values. The Applicant states that “[a] closer examination of the nitrate data showed that for most of the locations, the nitrate value used in these calculations was set at half the LOQ due to nonquantifiable levels of nitrate” (p 77, Technical Dossier). The limit of quantification (LOQ) used in this compositional analysis was 125 mg nitrate / kg sample material (p 12, Annex 8), and subsequently, samples with a nitrate concentration below the LOQ were recorded as having a nitrate concentration of 62.5 mg/kg. This estimated value was then used during all analyses.

The limit of quantification used in this study appears unreasonably high. Codex guidelines state that when undertaking compositional analysis of key components, “the methods of analysis should be sufficiently sensitive and specific to detect variations in key components” (Codex, 2003). Similar spectrophotometric studies of vegetable nitrate content have shown quantification limits of 5 mg/kg (Ayaz et al., 2007). Carrying out the compositional analysis of AM04-1020 with a greater sensitivity for nitrate quantification would eliminate any uncertainty regarding the statistical significance of tuber nitrate levels in AM04-1020 and Kuras.

Lastly, the use of additional reference lines values in the compositional data should not be confused with the comparator - the comparator is both closely related and is the most closely related conventional parental type (EFSA, 2011b). It is grown in each replicate side by side with the GMO. Reference lines often provide fewer replicates and introduce noise into the statistical analysis that may mask subtle but important differences.

Historical and literature derived ranges for compounds, as used here, should be avoided because these are not easily independently verified or reviewed, and may not be representative of the conditions under which the developer has measured the test GMO.

Recommendation: The Applicant should be required to repeat nitrate quantification measurements on tuber samples using a greater sensitivity and thus, a lower limit of quantification. The applicant should also restrict comparisons to the near genotype comparator only and not include data for reference lines grown under different conditions, times, or localities.

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

### **2.1 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. AM04-1020 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 AM04-1020 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 AM04-1020 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.

## 2.2 Post-release monitoring

The Applicant states, “[t]he by-products of the AM04-1020 processing are used as any other starch potato processing by-products in animal feeding. No post-market monitoring of GM food and feed is required.”

This is inconsistent with Directive 2001/18/EC and Appendix III of the 2005 revised regulations of the Norwegian Gene Technology Act, as the by-products contain GMO material in question and may conceivably result in adverse effects, despite the assumption by the Applicant of no possible adverse effects due to familiarity. Specifically, monitoring of ongoing nucleotide-level changes in the transgene and possible off-target effects of the dsRNA should be described in the monitoring plan.

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.

## 2.3 Environmental monitoring

In the environmental monitoring plan proposed by the Applicant in Annex 25, the Applicant states: “[i]t is being proposed that for AM04-1020 potato case-specific monitoring is not warranted or required, since the conclusions drawn in the environmental risk assessment were

based on scientific studies as presented in Appendices 2 to 25. and did not rely on any assumptions.”(p. 4).

The Applicant misinterprets the use of the word very narrowly in ”assumption” as an expression of truth without evidence. However, the intended meaning within the Directive is that akin to an expression of truth without certainty, including full scientific certainty. Hence, the Applicant has used a very narrow definition of ”assumption” to support the contention that case-specific monitoring is not required. This is contrary to the intentions of the monitoring requirement in with Directive 2001/18/EC.

**Recommendation:** The Applicant should provide a monitoring plan that tests the assertions of safety made within the environmental risk assessment.

Further, the Applicant has not given sufficient details on how the monitoring plans will meet the stated objectives. Based on our analysis the proposed monitoring plans does provide sufficient detail to ensure that the main requirements of monitoring outlined in requirements of Directive 2001/18/EC and Council Decision 2001/811/EC, or Appendix III of the 2005 revised regulations of the Norwegian Gene Technology Act will be fulfilled.

**Recommendation:** The Applicant should be required to submit a monitoring plan containing the more specific details on monitoring objectives, e.g. monitoring design, areas to be monitored, which monitoring networks will be engaged, or how the data will be analyzed.

### **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 AM04-1020. The Applicant should therefore 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 emphasize that potatoes are grown extensively in Norway and have both a traditional and cultural value for the Norwegian people. It is also important to evaluate whether alternative options, have 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, stipulates that health and environmental effects borne in other countries, (where GMOs are grown) must also be taken into consideration. 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 for cultivation 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 AM04-1020 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's information that may be of scientific relevance. Such independent evaluation is essential to maintaining rigorous standards expected in scientific practice. 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 AM04-1020. 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. Further the monitoring proposed by the Applicant does not contain sufficient details to ensure the key provisions of monitoring under the or Appendix III of the 2005 revised regulations of the Norwegian Gene Technology Act or of Directive 2001/18/EC and Council Decision 2001/811/EC. 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 as is. 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 AM04-1020 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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