BUNDESMINISTERIUM FÜR GESUNDHEIT

WISSENSCHAFTLICHE BEGRÜNDUNG FÜR EIN IMPORTVERBOT VON GENTECHNISCH VERÄNDERTER KARTOFFEL (*Solanum tuberosum* L. line EH92-527-1, NOTIFIKATION C/SE/96/3501) IN ÖSTERREICH

ZUSAMMENFASSUNG

Mit Beschluss vom 2. März 2010 (2010/135/EU) hat die Europäische Kommission (EK) das Inverkehrbringen dieser Kartoffel zugelassen, welche zur Erzielung eines höheren Amylopektin-Gehalts der Stärke gentechnisch verändert wurde (auch „Amylopektin-Stärkekartoffel“ genannt). Die Genehmigung erstreckt sich gemäß Art. 3 der Entscheidung der Kommission auf das Inverkehrbringen für den Anbau und für industrielle Zwecke. Am 31. März 2010 erteilte die zuständige Behörde Schwedens die endgültige Marktzulassung des Produkts (JORDBRUKSVERKET 2010). Am 10. April 2010 wurde diese gentechnisch veränderte Kartoffelsorte in den Gemeinschaftlichen Sortenkatalog der EU aufgenommen. Damit kann Pflanzgut dieser Kartoffel in der EU in Verkehr gebracht werden.

Sowohl am Regelungsausschuss am 4. Dezember 2006 als auch am Landwirtschaftsrat am 16. Juli 2007 sprach sich eine Reihe von Mitgliedstaaten gegen das Inverkehrbringen dieses Erzeugnisses für Zwecke des Anbaus aus, somit kam diese Marktzulassung für die meisten Mitgliedstaaten überraschend. Darüber hinaus hat der damals zuständige Umweltkommissar Dimas insbesondere wegen der schon damals vorhandenen Bedenken gegen das Antibiotikaresistenz-Markergen nptII dieses Vorhaben nicht weiter verfolgt und keinen Entscheidungsvorschlag vorgelegt. Dieses Gen vermittelt Resistenz gegen die Antibiotika Kanamycin, Neomycin und andere Aminoglykoside.

Hierzu ist auch auf Artikel 4 Abs. 2 der Freisetzungsrichtlinie 2001/18/EG hinzuweisen, demgemäß die Mitgliedstaaten und die Kommission dafür sorgen, dass GVO, die Gene enthalten, welche Resistenz gegen in der ärztlichen oder tierärztlichen Behandlung verwendete Antibiotika vermitteln, bei der Umweltsicherheitsbewertung besonders berücksichtigt werden; dies insbesondere im Hinblick auf die Identifizierung und schrittweise Einstellung von Antibiotikaresistenzmarkern in GVO, die schädliche Auswirkungen auf die menschliche Gesundheit oder die Umwelt haben können. Dies bedeutet, dass auch die Mitgliedstaaten verpflichtet sind, diesen Aspekt in der Risikobewertung besonders zu berücksichtigen.

Gemäß einer dazu auch vertretbaren Rechtsansicht gilt diese Bestimmung im Fall einer möglichen Vermittlung von Resistenz gegen human- oder veterinärmedizinisch bedeutsame Antibiotika sogar absolut und hätte diese schrittweise Einstellung gemäß Artikel 4 Abs. 2 bereits bis 31. Dezember 2004 erfolgen müssen. In diese Richtung geht bemerkenswerterweise auch der Appell von Kommissar Dalli im April 2010 an die Agrarbiotechnologie-Industrie, keine Antibiotikaresistenzmarker mehr zu verwenden.

In ihrem Gutachten vom 16. April 2004 hat die Europäische Lebensmittelsicherheitsbehörde (EFSA) die betreffenden Antibiotika als Therapeutika von untergeordneter Bedeutung in der Human- und Veterinärmedizin und damit auch die Verwendung von nptII als Markergen als unbedenklich eingestuft und diese Position in einem Statement im Jahr 2007 erneut bekräftigt (EFSA 2007). Stellungnahmen der WHO und der Europäischen Arzneimittelagentur EMEA im Zusammenhang mit der beantragten Zulassung der Amylopektinkartoffel belegen aber eindeutig die Wichtigkeit dieser Antibiotika (Kanamycin und Neomycin werden von der WHO als „highly important“ eingestuft) in der Veterinär- und Humanmedizin (EMEA 2007, WHO 2007). Im Jahr 2009 erfolgte im Auftrag der EK eine weitere Prüfung im Rahmen einer „Joint Scientific Opinion of the GMO and BIOHAZ Panels“ (EFSA 2009). Auch in dieser abschließenden Evaluierung erfolgte neuerlich eine positive Sicherheitsbewertung, allerdings eingeschränkt durch eine kritische „Minority Opinion“ von zwei Mitgliedern des BIOHAZ-Panels. Überdies wird in dieser konsolidierten Stellungnahme auf Unsicherheiten hingewiesen, die sich aus technischen Limitierungen bei der Probenahme, Detektion, bei der Abschätzung der Expositionsmengen und der Schwierigkeit, die tatsächliche Herkunft von Resistenzgenen zu definieren, ergeben (EFSA, 2009)

Österreich hat bereits im Rahmen des EU-Zulassungsverfahrens seine wissenschaftlichen Bedenken hinsichtlich der Sicherheit des Produkts für Mensch und Umwelt eingebracht und in der Folge die Meinung der EFSA kritisch hinterfragt, indem darauf hingewiesen wurde, dass z.B. die Annahme der EFSA, dass die natürliche Resistenzsituation gegenüber Kanamycin und anderen Aminoglykosiden in allen Ländern gleich (ubiquitär gleichmäßig verteilte, hohe Aminoglykosidresistenzraten) und somit der Anbau dieses Produkts unbedenklich sei, wissenschaftlich nicht aufrechterhalten werden kann.

In der vor drei Jahren fertig gestellten Studie „Risk Assessment of Antibiotic Resistance Marker Genes in GMOs“ (Wögerbauer 2007) konnte klar gezeigt werden, dass die natürliche Hintergrundbelastung mit Aminoglykosid-Resistenzen länderspezifisch stark variiert, die Bedeutung der inaktivierten Antibiotika national unterschiedlich gewichtet wird, eine niedrige horizontale Gentransferfrequenz zwischen Pflanze und Rezeptorbakterien keine Aussagen über negative Langzeitfolgen gestattet und dass das EFSA GMO Panel keinerlei quantitative Expositionsdaten für nptII zur Verfügung hat, um seine Annahmen seriös zu untermauern. Laut dieser Studie wäre somit auch eine eingehende Risikoabschätzung von nptII nach dem Fall zu Fall Prinzip vorzunehmen.

Österreich hat diese Kritik außerdem bereits bei der Zulassung der GVO Maislinie MON863 vorgebracht und eine Überprüfung durch die zu diesem Thema eingesetzte Arbeitsgruppe der Europäischen Kommission (GD Umwelt) verlangt. Diese Überprüfung ist bis dato nicht erfolgt.

Seitens der österreichischen zuständigen Behörde wurden darüber hinaus auch Bedenken gegen die Umweltverträglichkeitsprüfung sowie die in der Entscheidung angegebene Überwachung auf Auswirkungen auf die Umwelt und den von BASF vorgelegten Überwachungsplan vorgebracht.

Österreich sprach sich in der Folge in den zuständigen EU-Gremien gegen diese Produktzulassung aus.

Zusätzlich ist diese Stärkekartoffel auch im Verfahren gemäß der Verordnung (EG) Nr. 1829/2003 zur Zulassung als Lebensmittel und Futtermittel beantragt worden. In einem ebenfalls am 2. März 2010 erfolgten Beschluss der EK (2010/136/EU) wurde dieses Erzeugnis schließlich, wie von BASF beantragt, als Futtermittel, das aus dieser Kartoffelsorte gewonnen wird, zugelassen sowie auch als Lebens- und Futtermittel mit der Maßgabe, dass das Vorhandensein solcher Kartoffeln in Lebensmitteln oder Futtermitteln generell bis zu einem Anteil von 0,9 % zugelassen wird. Damit wird die der vorliegenden Entscheidung der EK für den Anbau vorgesehene Trennung der Warenströme erheblich unterwandert.

Zur Erhebung der Resistenzbelastung in Österreich wird die AGES beauftragt, dazu eine umfassende Studie durchzuführen, da insbesondere die Argumentation der EFSA, dass die bereits vorhandene Hintergrundresistenzbelastung einen zusätzlichen Resistenzgeneintrag via transgene Pflanzen egalisieren würde, quantitativ nicht belegt ist. Die in dieser Studie vorgesehene umfassende Dokumentation der Ausgangslage in Österreich in repräsentativen tier- und humanpathogenen Bakterienstämmen hinsichtlich der Häufigkeit des Vorkommens der entsprechenden Resistenzfunktionen soll eine verbesserte Abschätzung des Risikopotentials einer zusätzlichen Einbringung der Resistenzgene nptII und nptIII in den bereits vorhandenen Resistenzgenpool in Österreich ermöglichen. Diese Studie wird in etwa zwei Jahre in Anspruch nehmen.

Bis zum Vorliegen dieser Studie hält es das BMG im Sinne des Vorsorgeprinzips und auf Grund der wissenschaftlichen Unsicherheiten bei der Sicherheitsbewertung dieser gentechnisch veränderten Kartoffel angezeigt, ein bis zum 1. Dezember 2012 befristetes nationales Importverbot für das Inverkehrbringen zu Zwecken des Anbaus zu erlassen. Diese auf Grund des § 60 des Gentechnikgesetzes zu erlassende Verordnung wird dann entsprechend den Ergebnissen der Studie zu überprüfen sein.

Die Entscheidung durch den neu zuständigen Kommissar John Dalli war für die Mitgliedstaaten auch deshalb überraschend, da er und insbesondere Präsident Barroso vorher noch angekündigt hatten, die EK würde so rasch wie möglich eine rechtliche

Lösung vorschlagen, um den Mitgliedstaaten ein Selbstbestimmungsrecht für den Anbau von gentechnisch veränderten Pflanzen einzuräumen. In rechtspolitischer Hinsicht ist es daher auch zur Wahrung dieses von allen politischen Parteien, Landwirten und den Konsumenten geforderten Selbstbestimmungsrechtes eines Mitgliedstaates beim Anbau von GVO geboten, die gemäß der Richtlinie 2001/18/EG gegebene Möglichkeit eines entsprechenden Inverkehrbringensverbotes für den Anbau in Anspruch zu nehmen.

SCIENTIFIC ARGUMENTS FOR AN IMPORT BAN OF GENETICALLY MODIFIED POTATO (*Solanum tuberosum* L. line EH92-527-1, NOTIFICATION C/SE/96/3501) IN AUSTRIA

SUMMARY and BACKGROUND

The notification of genetically modified potato EH92-527-1 was originally submitted by Amylogene HB in 1996 under the provisions of Directive 90/220/EEC.
In 2003 an updated notification (Reference C/SE/96/3501) according to Directive 2001/18/EC concerning the placing on the market of genetically modified potato product (*Solanum tuberosum* L. line EH92-527-1) including a monitoring plan was submitted by Amylogen HB (now BASF Plant Science) to the competent authority of Sweden.
Originally the notification covered the placing on the market of *Solanum tuberosum* L. line EH92-527-1 for cultivation and processing into industrial starch, as well as use as feed in the Community.

The genetically modified product is described as follows (Art. 2 of COM 2010/135/EU):

1. The genetically modified organism to be placed on the market as or in products, hereinafter 'the product' is potato (*Solanum tuberosum* L.) modified for enhanced content of the amylopectin component of starch, which has been transformed with *Agrobacterium tumefaciens*, using the vector pHoxwG, resulting in line EH92-527-1. The product contains the following DNA in two cassettes:
(a) Cassette 1: a nptII-type kanamycin resistance gene originating from Tn5, under the regulation of a nopaline-synthase promoter for expression in plant tissue and terminated by a polyadenylation sequence from the *Agrobacterium tumefaciens* nopaline-synthase gene;
(b) Cassette 2: a segment of the potato gbss gene encoding for granule bound starch synthase protein inserted in reversed orientation under the control of the gbss-promoter isolated from potato, and terminated by a polyadenylation sequence from the *Agrobacterium tumefaciens* nopaline-synthase gene.
2. The consent shall cover genetically modified *Solanum tuberosum* L. line EH92-527-1 as or in products.

Following the notification procedure of Directive 2001/18/EC, the Swedish competent authority, that received the notification and was therefore responsible for the initial risk assessment, concluded that there is no scientific evidence to indicate that the placing on the market of the *Solanum tuberosum* L. line EH92-527-1 (GM potato EH92-527-1) poses any risk to human and animal health or the environment for the requested uses but stated also that it is very important that EH92-527-1 and products derived from this GMO are segregated from products intended for food use.

In 2005 the notifier excluded the use as feed from the scope of this notification, after Member States – like Austria - raised concerns during the notification procedure.

Subsequently the notifier submitted a notification according to Regulation (EC) No 1829/2003 covering the placing on the market of feed produced from GM potato EH92-527-1 and the adventitious or technically unavoidable presence of this GM potato in food and other feed products. Austria sent its opinion on the notification in September 2005 to EFSA containing the following criticism on the scope of this dossier:
„In the dossier according to Regulation (EC) No 1829/2003 on page 46, part I it is quoted "starch potatoes (altered starch content) are excluded from the OECD recommendation because they are not intended for human and animal consumption." Furthermore on page 48 it is quoted that these potatoes "are not intended for human consumption …[…]… and limited intake by animals via pulp" (is expected).
Therefore the use of the GMO for food and feed use as well as the use as food containing or consisting of this GMO should be excluded from the scope of this notification. If the scope of this (industrial) product is only extended up to food and feed use of the GMO because it "cannot be excluded that some (potato) may be present in food applications" (p. 46 of part I) then it cannot be regarded as acceptable that due to these facts this notification should cover the use as food. As a consequence also the use as feed should be scrutinized."

Moreover – due to Commission Decision 2010/136/EU – Austria is of the opinion that the mandatory segregation of this product[1] which is recommended according to the conditions for the approval according to Directive 2001/18/EC is actually foiled.

In 2006 the EFSA/GMO Panel published its Opinion on the placing on the market of GM potato EH92-527-1 for cultivation and industrial starch production under Directive 2001/18/EC (EFSA 2006a) as well as for food and feed under Regulation (EC) No 1829/2003 (EFSA 2006b) concluding that „for cultivation and industrial starch production under Directive 2001/18/EC and feed and food under Regulation (EC) No 1829/2003 the product is unlikely to have an adverse effect on human and animal health or the environment in the context of its proposed uses".

During the Regulatory Committee acc. to Directive 2001/18/EC on 4th December 2006 and at the Environmental Council on 16th July 2007 Austria – in line with a lot of other Member States – voted against the placing on the market of this product.

On 2nd March 2010 EC decided in favour of this product, which got final approval for placing on the market by the Swedish Competent Authority on 31st March 2010 (JORDBRUKSVERKET 2010). As a consequence, on 10th April 2010 this product was registered in the Common Catalogue of Varieties of Agricultural Plant Science.

[1] In this dossier it is quoted that "the notifier has created a system for separation, control and documentation… […] the notifier has committed itself, to make efforts to detect and track down potatoes if there is any suspicion that such potatoes have entered the food chain."

Austria still holds the opinion that the risks of this product on human and animal health as well as the environment were not assessed properly by the notifier and underlines this argumentation also with new, additional scientific arguments.
Additionally Austria will finance a two year field study aiming at:

1. Quantitative evaluation of the prevalence of the aminoglycoside resistance genes nptII and nptIII in naturally occurring bacterial populations. Establishment of the background levels of these antibiotic resistance determinants in natural habitats (determination of the "baseline").
2. Development of a model for the quantitative assessment of the impact of aminoglycoside phosphotransferases artificially introduced by GMOs into exposed bacterial populations. The data obtained during the determination of baseline will be used as reference level.
3. Determination of environmental conditions, which promote the formation of mosaic aminoglycoside phosphotransferase genes. Mosaic phosphotransferase genes may have aberrant substrate specificity with an inherent potential to inactivate aminoglycosides different from neomycin and kanamycin.
4. Analysis of the prevailing selection pressure in natural habitats, which may lead to the fixation of ARM genes in bacterial populations (using the data obtained in a concurrently performed project entitled "Antimicrobial consumption in Austrian livestock").

Intended results
1. Detailed quantitative information about the prevalence of the aminoglycoside resistance genes nptII and nptIII in natural habitats in Austria.
2. Establishment of a baseline concerning background antibiotic resistance rates (=frequency of nptII and nptIII genes in relevant habitats) before exposition of local ecosystems with ARM genes derived from transgenic plants. This baseline facilitates the detection of GMO induced aberrations in the prevalence of nptII and nptIII in certain bacterial populations after the artificially introduction of ARM genes.
3. The clarification whether aminoglycoside phosphotransferase genes show the potential to form mosaic gene structures will improve the risk assessment of ARM derived nptII and nptIII genes. The obtained data will provide information about their potential to expand their or the substrate specificity of other aminoglycoside phosphotransferases.
4. The data obtained from the proposed project will optimally supplement a currently ongoing project ("Antimicrobial consumption in Austrian livestock")

For the duration of this study as well as the quoted scientific reasons Austria has made use of the safeguard clause according to Art. 23 of Directive 2001/18/EC and prohibits the placing on the market for cultivation purposes of this product in Austria up to 1st December 2012. This timeframe should also be used for future scientific discussion on this GM potato EH92-527-1.

SCIENTIFIC ARGUMENTATION

1. Documents considered

The following reasoning is based on the documents provided by the notifier in the original application under Directive 2001/18/EC and the additional information received during the notification procedure as well as the parallel notification under Regulation (EC) No. 1829/2003 (for clarification purposes). Additionally, it takes into account the responses of the notifier to the statements of the Member States as well as the risk assessment report of the Swedish competent authority, the Scientific Committee of Plants and the European Food Safety Authority, EMEA (now EMA) and WHO as well as relevant literature.

2. Introduction

As described in chapter „Summary and Background“, the genetically modified product contains

a) a nptII-type kanamycin resistance gene
b) a segment of the potato gbss gene encoding for granule bound starch synthase protein

With regard to the nptII gene present in GM potato EH92-527-1 as selection marker, EFSA concluded in its Opinion that “the EFSA GMO Panel formulated already an Opinion (EFSA, 2004) on the use of antibiotic resistance genes in GM plants and concluded that the use of nptII as a selection marker did not pose a risk to the environment or to human and animal health”. This conclusion was based according to EFSA “on the limited use of kanamycin and neomycin in human and veterinary medicine, the already widespread presence of this gene in bacterial populations and the low risk of trans-kingdom gene transfer from plants to bacteria. EFSA further considered that nptII is a well-established selection marker with a history of safe use (Nap et al. 1992; Redenbaugh et al. 1994). This conclusion is consistent with earlier safety evaluations of nptII (SCP, 1998a)”, (all in EFSA 2004).
However, in 2007 neomycin and kanamycin were classified as “highly important antibacterials” by WHO (2007).
Additionally EMEA was consulted by the Commission regarding the authorisation of GM potato EH92-527-1 under Regulation (EC) No 1829/2003 and Directive 2001/18/EC and was contradicting the views presented by EFSA: EMEA pointed out the importance to consider a more long-term view in the risk assessment and underlined “the importance of neomycin and kanamycin as important therapeutics for human and veterinary medicine, concluding that their current and potential future use cannot be classified as of no or only minor therapeutic relevance” (EMEA 2007). Additionally EMEA addressed in its statement also other issues raised by EFSA rebutting their arguments.

Thereafter "the GMO Panel agreed with EMEA that the preservation of the therapeutic potential of the aminoglycoside group of antibiotics is important", but nevertheless re-affirmed in its additional statement its conclusions concerning the safety of nptII in its function as a selectable marker in genetically modified plants for human and animal health as well as the environment (EFSA 2007).
In 2007 a study on the "Risk Assessment of Antibiotic Resistance Marker Genes in Genetically Modified Organisms" (Wögerbauer 2007) was published, which was identifying a lot of deficiencies in the risk assessment of ARM-genes carried out so far, e.g. that it is not carried out on a case-by-case basis, and not taking into account local differences in resistance levels and antibiotic usage patterns.
On 7th May 2008 the College of the EU-Commission held an orientation debate on GMOs. The President convened this debate because this is a highly complex matter, subject to lively and often controversial debate in the Member States, which is currently evolving. With regard to this GM potato EH92-527-1 and three hybrid maize (MON863xMON810, MON863xNK603, MON863xMON810xNK603) – all these products contain the nptII gene - the Commission asked EFSA again to analyse further scientific evidence on the effects of these GMOs on the environment and human health.
This underlines the different opinions in the EC with regard to ARM-genes present in genetically modified plants and supports the Austrian arguments that the use of nptII as selectable marker in GMOs cannot be regarded as state of the art.

On 11th June 2009, EFSA published a „Joint Scientific Opinion of the GMO and BIOHAZ Panels" on the use of ARM genes in GM plants which concludes that the previous assessment of EFSA on GM potato EH92-527-1 is in line with the risk assessment strategy described in the statement, and that no new evidence has become available that would prompt EFSA to change its previous Opinion. However two Members of the BIOHAZ Panel expressed a critical minority Opinion on that issue. Additionally it has to be remarked that according to this Joint Opinion – „there are limitations related among others to sampling, detection, challenges in estimating exposure levels and the inability to assign transferable resistance genes to a defined source. The importance of taking these and other uncertainties described in this Opinion into account requires to be stressed" (EFSA 2009).

In this context it should also be taken into consideration, that according to Art. 4 of Directive 2001/18/EC "Member States and the Commission shall ensure that GMOs which contain genes expressing resistance to antibiotics in use for medical or veterinary treatment are taken into particular consideration when carrying out an environmental risk assessment, with a view to identifying and phasing out antibiotic resistance markers in GMOs which may have adverse effects on human health and the environment. This phasing out shall take place by the 31 December 2004 in the case of GMOs placed on the market according to Part C […]". As a consequence – according to this legal obligation and due to the recent scientific findings on nptII – this ARM-Gene should be phased out and the placing on the market of GMO-products containing this ARM-Gene should be prohibited.

A detailed scientific discussion of this topic is given under section 3 of this document.
In section 4 flaws in the molecular characterisation are described.
In section 5 monitoring of cultivation of GM potato EH92-527-1 is discussed.

Due to all these deficiencies in the risk assessment of this transgenic potato described and affirmed with new, additional scientific arguments, Austria has set into force a national safeguard measure for this product until 1st December 2012. This timeframe should also be used for a scientific discussion of the concerns against this GM potato EH92-527-1.

3. nptII-Gene

3.1. General remarks

In the following section the arguments of the notifier and their affirmation by EFSA on the safety of the nptII gene and the relevance on the market of the respective antibiotics for human and veterinary therapy are critically discussed.

EFSA has published three Opinions concerning the risk assessment of ARM genes in transgenic plants during the past six years and has found no objections against the application of nptII as marker gene in transgenic plants (EFSA 2004; EFSA 2007; EFSA 2009).

However, in our view the published EFSA Opinions are suffering from the following shortcomings:

1) Quantitative data concerning the prevalence of corresponding resistance determinants in natural habitats, which support the drawn conclusions, are missing. However, a quantitative understanding of the involved elements and processes is necessary to properly evaluate the impact of additionally introduced ARM genes into the environment.
2) The prevalence of antibiotic resistance determinants in bacterial populations is a dynamic phenomenon depending on the prevailing selection pressure in the habitat. Resistance gene prevalence may change from time to time. However, a prerequisite for a valid conclusion concerning the risk assessment of nptII is the availability of quantitative data concerning the amount of nptII homologues in the receiving environment and the number of ARM gene copies introduced into this environment.
3) Only the transfer of intact ARM genes has been considered in the official EFSA statements. The impact of resistance gene fragments, which may already carry mutations or introduce novel mutations during homologous recombination to complementary target sequences, has not been addressed. However such a mechanism should be taken into consideration since the substrate specificity of affected aminoglycoside phosphotransferases may be changed this way.

4) To our knowledge horizontal transfer frequencies of gene fragments mediated by natural genetic transformation in natural habitats have not been in the research focus, yet. Measuring these transfer frequencies is methodologically demanding and relies on sophisticated experimental designs. However, horizontal transfer of DNA fragments may be substantially more frequent compared to the frequencies established for the transfer of whole and intact resistance genes from transgenic plants.
5) A major factor for conclusions of EFSA on ARM genes appears to be the (quantitatively not defined) presence and broad distribution of (corresponding) resistance determinants in natural environments (EFSA 2004; EFSA 2007; EFSA 2009). However, a high prevalence of nptII genes in bacterial populations would provide a huge reservoir of potential partner molecules for homologous recombination with plant-derived bacterial ARM gene fragments resulting in the same effects as addressed under point 3.
6) The formation and impacts of mosaic aminoglycoside phosphotransferase gene structures upon uptake of nptII and nptIII fragments has not been considered in the risk assessment process for ARM genes.

In the most recent approach to assess the risks of ARM genes the GMO panel conceded uncertainties and limitations related to sampling, detection, challenges in estimating exposure levels and the inability to assign transferable resistance genes to defined sources (EFSA 2009). Two members of the BIOHAZ panel did not unequivocally share the final conclusions (EFSA 2009).

Therefore in our opinion the risk assessment of ARM genes performed by EFSA cannot be regarded as peremptory and decisive. The knowledge gaps described above and, for the associated uncertainties in the risk assessment of nptII are remaining. These gaps should be closed by providing missing quantitative data concerning the prevalence of ARM gene analogous resistance determinants in naturally occurring bacterial populations and by checking for environmental conditions supportive for the formation of mosaic aminoglycoside phosphotransferase genes before the risk assessment of nptII is finalized.

3.2. Therapeutic relevance of kanamycin and neomycin in human and veterinary medicine

NptII was classified by EFSA initially as group I ARM gene, which can be used without any restriction as selection marker in transgenic plants (EFSA 2004). A major factor leading to this classification was the assumption that the antibiotics inactivated by nptII are of low clinical relevance. However, the clinical relevance of antimicrobials is country specific and differs significantly throughout Europe (Wögerbauer 2007). In Austria a large number of neomycin containing drugs for human and veterinary applications are available (see Tables 1 – 2). Kanamycin is only used for veterinary purposes (see Table

3). Neomycin and kanamycin are, thus, important antibiotics for human and veterinary medicine in Austria.

Neomycin and kanamycin are both inactivated by the gene product of the aminoglycoside phosphotransferase nptII (=aph(3')-IIa), which phosphorylates the hydroxyl function at the C'-3 position of the aminoglycan ring (Mingeot-Leclercq et al. 1999). In Austria neomycin is applied for the topical treatment of skin and mucosal infections in humans (Österreichische Gesellschaft für Chemotherapie 2009). Currently, 12 drug preparations containing neomycin are licensed and available on the market (see Table 1) (Austria Codex 2009; Österreichische Gesellschaft für Chemotherapie 2009). Eleven neomycin preparations are available for veterinary applications. Predominantly neomycin is used for the treatment of mastitis and enteritis in cattle, pigs and poultry. Additionally neomycin preparations are used for treatment of eczema and dermatitis of microbial origin in pets (e.g. cats, dogs, etc...), (see Table 2).

In Austria kanamycin is of no significance for the treatment of infectious diseases in humans (Österreichische Gesellschaft für Chemotherapie 2009). Nevertheless, this aminoglycoside is an important second line antibiotic for the treatment of multidrug resistant tuberculosis (especially for the extremely difficult therapy of infections with XDR Mycobacterium tuberculosis) in the Anglo-American hemisphere (Canada, USA) (EFSA 2009; Horsburgh, C. R., and W.J. Burman 2003; Van Deun et al. 2010). In Austria three kanamycin preparations are licensed for treatment and prophylaxis of a broad range of infections in cattle, sheep, pigs and poultry (see Table 3).

Amikacin, a semi-synthetic derivative of kanamycin A, is a crucially important aminoglycoside antibiotic exclusively restricted for the treatment of serious infections in humans. Amikacin is applied for the treatment of bacterial strains, which have been proven to be resistant to other classes of aminoglycoside drugs (e.g. gentamicin) (Horsburgh, C. R., and W.J. Burman 2003; Österreichische Gesellschaft für Chemotherapie 2009). Moreover, amikacin is an important second line antibiotic for the treatment of multidrug resistant tuberculosis (Horsburgh, C. R., and W.J. Burman 2003; Österreichische Gesellschaft für Chemotherapie 2009). There is no indication for amikacin application in veterinary medicine (EFSA 2004). Amikacin is inefficiently phosphorylated by NPTII (Perlin, M. H., and S. A. Lerner 1986). A clinically relevant resistance phenotype can only be observed if the nptII carrying bacterial host suffers from additional mutations (Perlin, M. H., and S. A. Lerner 1986). Amikacin is an optimal substrate for NPTIII (Mingeot-Leclercq et al. 1999).

Amikacin has been classified as "critically important antibiotic" by a WHO expert working group; neomycin and kanamycin were characterized as "highly important" antimicrobials (WHO 2007). EMEA is also of the opinion that neomycin and kanamycin continue to play a role in clinical and veterinary applications (EMEA 2007).

Due to a constant increase of bacterial strains resistant to various different classes of antibiotics during the past decades one may also have to face the fact that some terminal cases will have to rely on a treatment with older (aminoglycoside) antibiotics with unfavourable pharmacologic properties in the future (EMEA 2007).

Neomycin: Relevance for clinical applications in Austria

Preparation	Active Ingredient	Marketing Authorisation Holder	Indications[1)]
Baneocin pro instillatione	Neomycin Sulfate Bacitracin (20:1)	Sandoz GmbH Kundl, Austria	instillation as lavage or as aerosol inhalation, lavage for fistula, for surgical interventions; sinusitis, otitis media, wound infections; aerosol inhalation as supplemental therapy for upper respiratory tract infections
Baneocin powder	Neomycin Sulfate Bacitracin (20:1)	Sandoz GmbH Kundl, Austria	topical applications; skin infections (small areas), prophylaxis for umbilical infections; after surgical interventions (skin)
Baneocin ointment	Neomycin Sulfate Bacitracin (20:1)	Sandoz GmbH Kundl, Austria	topical applications; focal bacterial skin infections: furunclosis, carbuncle (after surgical treatment), folliculitis barbae, folliculitis profunda, hidradenitis suppurativa, periporitis, paronychia; impetigo contagiosa, infected ulcera cruris, secondary infections of eczema
Betnesol N eye, ear and nosedrops	Betamethason Neomycin Sulfate	Defiante Farmaceutica SA Funchal, Madeira (PT)	eye: non-infected inflammatory diseases, at risk for bacterial infections ear: otitis externa and other inflammatory diseases with manifest or expected bacterial infection nose: inflammatory diseases at risk for bacterial infections
Betnovate N creme	Betamethason Neomycin Sulfate	GlaxoSmithKline Pharma GmbH, Vienna, Austria	bacterial secondary infections of the skin with pathogens sensitive to neomycin:

Preparation	Active Ingredient	Marketing Authorisation Holder	Indications[1)]
			• eczema: children (from 2 years) and adults (including atopic and discoid eczema) • prurigo nodularis • psoriasis (except for psoriasis with extended Plaques) • seborrhoic dermatitis • contact dermatitis • intertrigo analis und genitalis
Betnovate N ointment	Betamethason Neomycin Sulfate	GlaxoSmithKline Pharma GmbH, Vienna, Austria	see Betnovate N - creme
Hydoftal 1,5 % eye ointment	Hydrocortisone, Hydrocortisone 21-acetate, Neomycin Sulfate	AGEPHA, Söding, Austria	blepharitis (non purulent) conjuctivitis (non purulent) especially if cause by allergy keratitis without defect of the epithel (do not apply with Sjögrenschic kerato-conjunctivitis and yperite induced keratitis iritis, iridozyclitis, scleritis, episkleritis postsurgical non-contagious irritations
Hydoftal 2,5 % eye drops	Hydrocortisone Neomycin Sulfate	AGEPHA, Söding, Austria	see Hydoftal 1,5 % - eye ointment
Nebacetin pro instillatione	Neomycin Sulfate Bacitracin	Sandoz GmbH Kundl, Austria	instillations and/or lavages with head and neck diseases, surgical, urologic, dermatologic and ophthalmologic applications; inhalation of aerosols with respiratory diseases
Nebacetin powder	Neomycin Sulfate Bacitracin	Sandoz GmbH Kundl, Austria	bacterial skin and small superficial wound infections and their prophylaxis; burns, scalding; Herpes zoster and H. simplex induced blisters with bacterial infections; follow up treatment of perineal lacerations, episisotomy, mastitis after incisions, prophylaxis of

Preparation	Active Ingredient	Marketing Authorisation Holder	Indications[1)]
			mastitis an umbilical infections, bacterially induced diaper dermatitis
Nebacetin ointment	Neomycin Sulfate Bacitracin	Sandoz GmbH Kundl, Austria	topical application; focal bacterial skin infections: furunclosis, carbuncle (after surgical treatment), folliculitis barbae, folliculitis profunda, hidradenitis suppurativa, periporitis, paronychia; impetigo contagiosa, infected ulcera cruris, secondary infections of eczema
Otosporin ear drops	Neomycin, Polymyxin B, Hydrocortisone	GlaxoSmithKline Pharma GmbH, Vienna, Austria	topical applications: bacterial infections of the external auditory canal

Table 1: Available neomycin preparations in Austria (as of: March 2010) (Austria Codex 2009; Österreichische Gesellschaft für Chemotherapie 2009)
[1)] Product information from the manufacturer

Neomycin: Relevance for veterinary applications in Austria

Preparation	Active Ingredient	Marketing Authorisation Holder	Indications[1)]
Ani-Neopre drug premix for feed applications for pigs and calves	Neomycin Sulfate	Animed Service AG Graz, Austria	therapy and supplemental live stock treatment of bacterially induced diseases of the gastrointestinal tract: enteritis caused by E. coli, Salmonella, Pasteurella etc…for piglets and non-ruminating calves; oedema disease; MMA complex of breeding sows
Cloxagel forte injectors for animals	Cloxacillin Benzathin, Neomycin Sulfate	Virbac Laboratoires, Carros, France	prophylaxis and therapy of mastitis for cattle at the dry stage
Cloxagel injectors for animals	Natrium Cloxacillinat, Neomycin Sulfat	Virbac Laboratoires, Carros, France	treatment of mastitis for lactating cows
Enteran powder for animals	Colecalciferol, Neomycin Sulfat, Phthalylsulfathiazol, Retinol, Sulfadimidin, Tanninum albuminatum	aniMedica GmbH, Senden-Bösensell, Germany	calves, pigs and piglets with infectious diarrhoea caused by neomycin sensitive bacteria
Lincocin forte solution for intramammary application in cattle	Lincomycin Hydrochloride, Neomycin Sulfate	Pfizer Corporation Austria GmbH, Vienna Austria	Inflammation of the udder induced by lincomycin and neomycin sensitive pathogens; for lactating cows
Mastitar udder injector for cattle	α-Tocopherolacetat, Benzylpenicillin Kalium, Neomycin Sulfate, Procain Benzylpenicillin	Virbac Laboratoires, Carros, France	for drying of a clinically healthy udder of dairy cows taking into account the clinical condition of the udder and the resistance pattern of the herds

Preparation	Active Ingredient	Marketing Authorisation Holder	Indications[1)]
Neo-Mix drug premix for feed applications for animals	Neomycin Sulfate	AniMed Service AG Graz, Austria	therapy and supplemental live stock treatment of bacterially induced diseases of the gastrointestinal tract: enteritis caused by E. coli, Salmonella, Shigella, Pasteurella, Vibrio etc… pigs: diarrhoea, oedema disease, salmonellosis, MMA complex of sows hens and turkeys: Vibrio induced hepatitis, enteritis induced by E. coli, salmonellosis
Neo-Mix powder for feeding animals	Neomycin Sulfate	AniMed Service AG Graz, Austria	see Neo-Mix - drug premix for feed applications for animals
Neomycin-Penicillin injectable suspension for pigs and cattle	Neomycin Sulfate, Procain Benzylpenicillin	Intervet GmbH, Vienna, Austria	infectious diseases induced by multiple penicillin and neomycin sensitive bacterial pathogens (especially E. coli) rearing diseases of calves and piglets, coli-induced bacillosis, MMA complex of sows mastitis, endometritis of cows
Neomycinsulfat "Chevita" 70 % powder for animals	Neomycin Sulfate	Chevita Tierarzneimittel - Gesellschaft m.b.H., Wels, Austria	for the treatment of bacterially induced enteritis caused by neomycin sensitive E. coli, Salmonella or Vibrio strains
Panolog ointment for dogs and cats	Neomycin Sulfate, Nystatin, Thiostrepton, Triamcinolon Acetonid	Novartis Animal Health GmbH, Kundl, Austria	for the treatment of acute and chronic otitis externa, interdigital eczema, inflamation of anal glands; therapy of inflammatory dermatosis, dry and exsudative dermatitis in combination with bacteria or mycotic infections, eczema

Table 2: Neomycin preparations for veterinary applications available in Austria (as of: March 2010). Information about veterinary medicinal products in Austria, Federal Ministry of Health, Austria. Published: 17/04/2009.

http://www.bmgfj.gv.at/cms/site/standard.html?channel=CH0723&doc=CMS1216820062496

[1)] Product information from the manufacturer

Kanamycin: Veterinary applications in Austria

Preparation	Active Ingredient	Marketing Authorisation Holder	Indications[1)]
Kanamycin "Virbac" puncture bottle for animals	Kanamycin Sulfate	Virbac Laboratoires, Carros, Frankreich	contagious organ and general diseases caused by pathogens sensitive to kanamycin; for cattle, sheep, pigs, poultry
Ubrolexin suspension for intramammary application with lactating cows	Cefalexin, Kanamycin Sulfate	Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Deutschland	for the treatment of clinically apparent mastitis caused by pathogens sensitive to kanamycin and cefalexin
Vanakan 10% injection solution for animals	Kanamycin Disulfate	Vana GmbH, Vienna, Austria	therapy and prophylaxis of infectious diseases caused by pathogens sensitive to kanamycin for cattle, sheep, pigs, poultry

Table 3: Kanamycin preparations for veterinary applications in Austria (as of: March 2010).

[1)] see table 2

3.2.1. Resistance to kanamycin/neomycin in clinical isolates

Resistance to kanamycin or neomycin is well known in clinical environments, although the frequency is varying considerably between locations and bacterial strains, possibly reflecting varying selection pressure. NptII was shown to be located on the transferable transposon Tn5 (Beck et al. 1982) and, thus, is supposed to be easily spread within bacterial communities via conjugation. However, phenotypical resistance to kanamycin is only rarely mediated by nptII in clinical isolates (Shaw et al. 1993). In theses rare cases Pseudomonas, Aeromonas and Escherichia coli have been shown to be major nptII carriers (Smalla et al. 1993). Alvarez et al. demonstrated a low prevalence of nptII genes in clinical samples (Alvarez, M., and M. C. Mendoza 1992).

In Austria resistance of major pathogens like *Salmonella sp*. and *Campylobacter sp.* to kanamycin is usually rare and/or clinically insignificant (Mittermayer 2007).

3.2.2. Resistance to kanamycin/neomycin in environmental samples of animal origin

Variable resistance rates of bacterial isolates of animal origin to kanamycin are reported in the scientific literature (Brun et al. 2002; Gibreel et al. 2004; Hauschild et al. 2007; Novais et al. 2005; Poeta et al. 2007; Schmitz et al. 1999; Travis et al. 2006). Clinically irrelevant resistance to neomycin was found in *Enterococcus faecalis* isolates (2-3%;

isolated from poultry faeces) (Nielsen et al. 2005). For Scandinavia no study is available which is dealing systematically with the prevalence of nptII in environmental samples, although phenotypic resistance to kanamycin is relatively common (Nielsen et al. 2005). A study analyzing the resistance profiles of *Campylobacter jejuni* from wild bird populations could not detect any kanamycin resistant isolates. This can be an indication that birds are presumably no reservoir for nptII genes (Waldenstrom et al. 2005).

In Austria phenotypic kanamycin resistance of bacterial strains obtained from animals is usually rare (Mittermayer 2007).

3.2.3. Resistance to kanamycin/neomycin in soil samples

A thorough survey of the scientific literature retrieved only 3 publications, which were dealing explicitly with the prevalence of nptII genes in natural environments (Leff et al. 1993; Smalla et al. 1993; Zhu 2007).
Two of these document a low-level presence of nptII in naturally occurring bacterial populations of non-clinical origin. Leff et al. could detect nptII only in samples from river water (3 out of 184 isolates), Smalla et al. found nptII positive isolates in sewage, and manure (3 out of 350). It must be stressed that resistance rates of approx. 3% and 1%, respectively, are clinically irrelevant. Both studies were performed in 1993 and due to the applied methodology semi-quantitative at best.

A more recent study by Zhu (2007) analyzed samples from Canada for the presence of nptII in river water. The analyses conducted over a 2 years period showed extremely variable nptII concentrations ranging from 0 to 4,36 x 10^6 copies per litre of water. The experimental setting was not representative for the relevant European conditions and environmental exposure pathways (GMO exposed soil and/or gut bacteria).

Up to 10^5 bacteria expressing a kanamycin resistant phenotype per gram of soil are usually detectable in various soil samples (Smalla et al. 1993). Concerning the analysis of soil samples one has to bear in mind that usually only a minor fraction of approx. 1 % of the bacteria can be cultivated under laboratory conditions (Demanèche et al. 2008).

There are no up to date data available concerning the prevalence of nptII in soil habitats in Austria and throughout Europe.

3.3. The aminoglycoside phosphotransferase gene aph(3')-IIa as a risk factor

Horizontal transfer of plant-derived ARM genes to soil or gut bacteria resulting in an impaired antimicrobial treatment of animal and human infectious diseases is unlikely but cannot be excluded a priori (EFSA 2009; Gay, P. B., and S. H. Gillespie 2005; Goldstein et al. 2005). However, the additional input of DNA which is coding for antibiotic resistance functions derived from transgenic organisms over extended periods of time via plant decaying processes or uptake by food or feed would certainly increase

the likelihood for contacts between resistance encoding DNA and competent bacteria (Nielsen et al. 2005). Comparing the situation with mosaic penicillin or tetracycline resistance genes (Smith et al. 1991; for details see section 3.4.), where the donor DNA most likely originates from surrounding dead bacterial cells, DNA from decaying plant material may provide a quantitatively similar environment for DNA uptake via natural genetic transformation.

At present it is unclear whether this increased exposure rate is actually capable to induce changes in the composition of the global antibiotic resistance gene pool. According to the consolidated scientific Opinion of the EFSA GMO and the BIOHAZ panel an increased failure rate during the treatment of infectious diseases directly attributable to disseminated ARM genes is unlikely (EFSA 2009). However, a quantitative analysis or other direct experimental evidence backing this conclusion is lacking. Additionally, the authors identify a certain degree of uncertainty (like limitations related to sampling, detection, challenges in estimating exposure levels and the inability to assign transferable resistance genes to a defined source), which have had to be considered during the preparation of the Opinion (EFSA 2009). Two members of the BIOHAZ panel did not concur with the final consolidated conclusion and expressed minority Opinions concerning the likelihood of adverse effects of ARM genes (nptII, aadA) to human health and the environment (EFSA 2009).

The neomycin phosphotransferase nptII (= aph(3')-IIa) inactivates the aminoglycoside antibiotics kanamycin, neomycin, paromomycin, ribostamycin, butirosin, gentamicin B and geneticin (= G418) by phosphorylation of the hydroxyl at position C3 in the first aminoglycan ring (Mingeot-Leclercq et al. 1999). In vitro inactivation of amikacin was reported, but only a combination of a chromosomal mutation, which reduces the general uptake of aminoglycosides and a second mutation, which increases the copy number of the plasmid carrying the aph(3')-IIa gene, leads to high level amikacin resistance (Perlin, M. H., and S. A. Lerner 1986).
The nptII variant currently used in transgenic plants inactivates only kanamycin, neomycin and geneticin (Redenbaugh et al. 1993; Redenbaugh et al. 1994).

It is supposed that natural environments harbour resistance determinants inactivating kanamycin and neomycin to a large extent and that DNA transfers from plant to bacteria are extremely rare events under naturally occurring conditions (EFSA 2009). According to the most recent Opinion of the EFSA GMO Panel, however these are the two major reasons why an additional external input of nptII into local ecosystems may not be clinically apparent (EFSA 2009).

However, this conclusion is based upon experiments with marker gene rescue systems which rely on restoration of function of a mutated or truncated endogenous version of the nptII gene by replacement via an intact full-length, plant derived aph(3')-IIa gene (de Vries, J., and W. Wackernagel 1998; Nielsen et al. 2000). To our knowledge

transformation and recombination frequencies of ARM gene derived DNA fragments have not been considered, yet.

In our opinion the following facts have not been taken adequately into account:

- The plant derived nptII gene suffers from the plant specific endogenous mutation rate (e.g. maize: 10^{-8}/1000 bp/generation). Point mutations introduced into ARM genes during plant cell replication may change the substrate specificity of the aminoglycoside phosphotransferase if effectively transferred to bacterial recipients.
- The horizontal transfer of ARM gene fragments was not considered sufficiently. Only the uptake of full-length, intact nptII genes was evaluated. The uptake of nptII fragments and a subsequent chromosomal integration into an already present copy of nptII may result in sequence alterations induced by homologous recombination or may lead to the formation of mosaic genes, which may change substrate specificity of the endogenously present aminoglycoside phosphotransferase.
- Large scale cultivation and long term exposure over generations (food/feed) increase the possibility of contacts between ARM gene encoding DNA and competent bacteria.
- A massive additional input of artificial plant-derived resistance encoding determinants into the environment of bacteria will increase the potential for recombination and, thus, the chance for formation of new variants of aminoglycoside phosphotransferases.

3.4. Mosaic genes

Homologous recombination is a cornerstone for bacterial diversity and evolution and is not restricted to the transfer of intact functional genes but may also facilitate the integration of shorter DNA fragments into microbial genomes (Didelot, X., and M. C. Maiden 2010; Smith et al. 1991).

Transfer of free resistance-encoding DNA fragments followed by recombination with homologous sequences in the receptor cell is a well-established process (Dowson et al. 1994; Smith et al. 1991). DNA from decaying plant material encoding bacterial resistance determinants may be a similar effective source of DNA-fragments as DNA from disrupted bacterial cells.

The relevance of the process relying on the recombination of external DNA fragments internalized via natural genetic transformation is illustrated below for two representative examples (mosaic genes for penicillin binding proteins and tetracycline resistance determinants):

A crucial mechanism responsible for the development of resistance to new classes of beta lactams in *Streptococcus pneumoniae* is based upon the formation of new variants of penicillin binding proteins (e.g. pbp / pbp 2x) (Claverys et al. 2000). These genes are composed of DNA fragments originating from different bacterial species. *S. mitis* and *S. oralis* could be demonstrated to have been the major donors of these fragments which have been subsequently transferred to *S. pneumoniae* via natural genetic transformation. Homologous recombination with complementary endogenous gene sequences resulted in mosaic genes coding for new pbp proteins with altered substrate specificity (Dowson et al. 1994). A similar phenomenon was described with penicillin binding proteins (penA) in *Neisseria gonorrhoeae*. The non-pathogenic *Neisseria N. flavescens* and *N. cinera* were identified to be the reservoir for DNA fragments encoding penicillin resistance. Upon uptake by *N. gonorrhoeae* and recombination with already present homologous resistance functions, a patchwork of external and endogenous penA DNA fragments form a new mosaic penA resistance gene with an altered substrate spectrum (Bowler et al. 1994). Only recently a high prevalence of tet(O) and tet(W) mosaic genes in feces of humans, pigs, and in probiotics (*Lactobacilli*, *Bifidobacteria*) mediating resistance to a broad range of tetracycline has been reported (Patterson et al. 2007; van Hoek et al. 2008).

Before drawing a final conclusion on the risk potential of nptII it should be investigated whether aminoglycoside phosphotransferase genes have a similar potential to form mosaic gene structures via uptake of DNA fragments and recombination with endogenously present aph sequences like penicillin binding protein and tetracycline resistance genes. It may be also of interest to check whether aph(3')-IIa fragments have the ability to modify endogenously present sequences of other aminoglycoside phosphotransferase genes. The clarification of this issue is of immediate interest for assessing the potential for changing the substrate specificity of the involved aminoglycoside phosphotransferases.

4. Molecular characterisation

4.1. General remarks

For risk assessment of this GM potato EH92-527-1 the data package submitted under C/SE/96/3501 was evaluated. However, to solve inconsistencies and to look for missing information from the C-dossier C/SE/96/3501 also material from notification EFSA-GMO-NL-2009-14 was assessed. As notification EFSA-GMO-NL-2009-14 was the last package submitted by the notifier to EFSA it is supposed to contain the data from C/SE/96/3501 and additionally the most up-to-date and comprehensive data package available for the risk assessment of GM potato EH92-527-1.
Nevertheless, the notification EFSA-GMO-NL-2009-14 according to Regulation EC No. 1829/2003 suffers from several shortcomings exacerbating a conclusive final risk assessment concerning the molecular characterization of GM potato EH92-527-1.

The major points of criticism concerning this notification are summarized below:

- No sequence information 3' downstream of the genomic insertion locus flanking the transgenic insert is available.
- No data concerning the insert copy number are presented in notification EFSA-GMO-NL-2009-14. The notification according to Directive 2001/18/EC contains conflicting information on this issue based upon Southern blot data of inferior quality. Tetraploidity of the potato genome is not considered in this respect.
- The molecular mechanisms causative for the downregulation of endogenous gbss expression are not discussed. The initially unintended effects resulting from the substantial rearrangement of the transgenic insert after genomic integration are not considered. Concerning the molecular interactions leading to the silencing of the endogenous gbss gene EH92-527-1 is a „black box".
- The presentation of the data in the notification EFSA-GMO-NL-2009-14 is sloppy (wrong restriction enzyme target sites in sequence annotations, no designation of genetic elements to the corresponding sequences, discrepancies in vector lengths and descriptions between technical dossier and raw data presented in the respective annexes) and relying on Southern blot analyses of poor quality (intense background staining, smears instead of distinct banding patterns, signal intensities of positive controls covering substantial areas of the whole blot, inappropriate choice of molecular weight markers, which do not allow a proper determination of the length of target bands, probe hybridisations in lanes containing no targets etc...).
- The displayed SDS-PAGE photos do not show the whole gel and suffer from substantial overloading of important sample lanes. A different protein banding pattern between EH92-527-1 and non-GMO controls is ignored. The putative GBSS fragment is not verified via immunostaining in a Western blot.
- No data on the gene expression of the introduced genetic modification (= antisense gbss gene in the transgenic insert) has been submitted. The test item is

the transcribed antisense gbss pre mRNA. Quantitative data on antisense gbss pre mRNA levels are missing. These data are required according to the relevant EFSA guidance document (EFSA 2006c).

- Genetic and phenotypic stability of the transgenic insert is not unequivocally established in notification EFSA-GMO-NL-2009-14. The notifier only presents amylose contents over a test period of 4 years changing the methodology during the test period to an amylose insensitive procedure. The data indicate a substantial decrease of the amylose content to 26% if compared to the initial amount at the beginning of the test period. These data are ineligible to provide evidence for a stable gene expression.
- Additional information concerning the genotypic stability presented according to Directive 2001/18/EC fails to indicate the number of individual plants used for sequencing. The results reported in the additional clarifications do not cover the whole sequence
- At several occasions the notifier has refused to provide additional experimental data requested by EFSA and/or national Competent Authorities referring to available data being substandard at best (Southern blots) or to technological problems, which would have had required some more sophisticated input by the applicant (sequencing of the 3' flanking genomic region) and, thus, he appeared to be uninterested in solving issues concerning basic aspect of the risk assessment of GM potato EH92-527-1.

These shortcomings increase the degree of uncertainty in the risk assessment of GM potato EH92-527-1 and of the final EFSA Opinion.

We would also like to draw the attention to the fact that the genetic organisation of the transgenic insert actually present in the GM potato EH92-527-1 does not correspond to the description of the product set out in Art. 2 of Commission Decision COM 2010/135/EU (see Table4).

Genetic elements in GM potato EH92-527-1		
According to Commission Decision **COM 2010/135/EU**		Actual conformation in GM potato EH92-527-1 according to Notification C/SE/96/3501//EFSA-GMO-NL-2009-14
Cassette 1	1 x nos promoter	**2** x nos promoter
	1 x nptII	**2** x nptII
	1 x nos terminator	**2** x nos terminator
Cassette 2	1 x gbss promoter	**2** x gbss promoter
	1 x gbss fragment	**2** x gbss fragment
	1 x nos terminator	**Missing**
Explanation	Represents intact transformation vector pHoxwG.	Truncation of cassette 2, duplication of intact cassette 1 and truncated cassette 2; annealing of both cassettes in an inverted repeat tail-to-tail rearrangement upon chromosomal integration

Table 4. Conformation of the transgenic insert in GM potato EH92-527-1

The shortcomings concerning points 1 - 9 are detailed below (see sections 4.2 - 4.11).

4.2. Nature and source of vector used

The description of the transformation vector pHoxwG by the notifier is inconsistent:

- The denoted length of the plasmid pHoxwG in Figure 2 of the technical dossier of notification EFSA-GMO-NL-2009-14 (= 15626 bp) does not correspond to the number of base pairs denoted to the pHoxwG DNA sequence in Annex 3 (= 15202 bp) (= Annex 1; C/SE/96/3501). The notifier does not provide an explanation for the discrepancy of the missing 424 bp vector sequence.
- The numbering system and the displayed restriction enzyme target pattern used in Figure 2 of the technical dossier do not correspond to the numbering and restriction scheme in Annex 3. Obtaining sequence information for the genetic elements displayed in Figure 2 is hampered.
- The restriction sites in Annex 3 are designated to the wrong target sequences. Obtaining sequence information for the genetic elements displayed in Figure 2 is hampered.
- The notifier omitted an allocation of the distinct genetic element to the corresponding sequence in Annex 3.
- This resulted in the impossibility to allocate the first genetic element of 372 bp described in Table 1 of the technical dossier to a corresponding sequence of the pHoxwG vector described in Annex 3. Of the second element of Table 1 designated to contain 983 bp of Tn5 and nptII fragments only nptII (794 bp) can be allocated unequivocally to the vector sequence presented in Annex 3.

- The GBSS promoter sequence of vector pHoxwG presented in Annex 3 does not correspond to the sequence A23740 deposited in Genbank and in the initial patent claim "WO 9211376-A". The displayed pHoxwG GBSS promoter sequence contains five alterations namely 4 single nucleotide insertions and one single nucleotide deletion. The notifier does not discuss the cause and effects of these alterations.
- The length of the GBSS promoter sequence is indicated to be of 987 bp, however the sequence denoted in Table 1 of the technical dossier contains 989 bp.
- The polyadenylation sequence from the nopaline synthase gene is indicated to be of 252 bp, however Table 1 designates 255 bp to this genetic element.

In summary this unreliable description of the transformation vector impedes a proper evaluation of the transgenic insert in GM potato EH92-527-1. This circumstance increases the degree of uncertainty in the risk assessment of GM potato EH92-527-1 and of the final EFSA Opinion.

4.3. Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion

The intended function (= inactivation of the endogenous gbss mRNA by vector encoded antisense gbss pre mRNA) has been demonstrated only indirectly by a simple SDS-PAGE showing the disappearance of an undefined smear (Figure 3, Annex 8, EFSA-GMO-NL-2009-14) in the range of approx. 56 to 76 kDa in the lane containing material from EH92-527-1. The notifier does not provide evidence that the smear represents endogenous GBSS. Using gel photos of inferior quality and uncharacterized protein bands in the line of argumentation as proof of principle exacerbates the risk assessment of GM potato EH92-527-1 and increases the degree of uncertainty of the final EFSA Opinion.

4.4. Description of the trait(s) and characteristics which have been introduced or modified

The interactions of the introduced trait (antisense gbss gene) with its targets are not elaborated. The sole statement from the notifier concerning this issue is that the "…gbss gene from *Solanum tuberosum* in antisense relative to the gbss promoter leads to a decrease in amylose content … in tuber starch … of EH92-527-1." The molecular mechanisms of interaction which mediate gene silencing are not described in the technical dossier (EFSA-GMO-NL-2009-14). The immediate and unintended effects due to the formation of extensive intramolecular double stranded RNA regions on gene silencing induced by the actual insert conformation, which was the result of a substantial rearrangement during insertion into the potato genome, are not considered. According to the data presented by the notifier concerning the molecular effects leading to the reduction of endogenous gbss gene expression EH92-527-1 must be rated as a "black box". This observation is disconcerting, impedes the risk assessment of GM potato EH92-527-1 and increases the degree of uncertainty of the final EFSA Opinion.

4.5. Information on the sequences actually inserted or deleted

The transgenic insert of GM potato EH92-527-1 suffered a substantial rearrangement and now consists of two nptII expression cassettes and a duplicated gbss element in form of an inverted repeat in a tail-to-tail structure with two right border regions as junctions to the potato chromosomal DNA. The gbss containing expression cassettes have lost their NOS terminators. These alterations lead to the following consequences:

1) Transcription from this DNA template lacking transcription terminator sequences results in the production of antisense gbss pre-mRNAs with undetermined 3' ends.
2) There is the possibility of convergent transcription from the two opposing promoters leading to colliding RNA polymerase elongating complexes – a process which is likely to truncate the pre-mRNAs at unspecified positions.
3) The transcribed antisense gbss pre-mRNA contains an extensive region of sequence complementarity prone to form intramolecular double stranded RNA duplexes.
4) This intramolecular double stranded RNA region induces the formation of siRNA silencing complexes (Yan, 2006), which have never been planned or intended by the notifier according to the applied transformation vector pHoxwG.

Considering points 1 – 4 the notifier did not provide any data to characterize the antisense gbss RNA molecules produced from the transgenic insert nor did he provide any information on the mechanisms underlying the silencing of the endogenous potato gbss gene. The actual conformation of the transgenic insert and the resulting induction of siRNAs are a chance hit never intentionally planned by the developers.
This exacerbates the risk assessment of this application and increases the degree of uncertainty of the final EFSA Opinion.

4.6. The size and copy number of all detectable inserts, both complete and partial

Concerning the notification of GM potato EH92-527-1 according to Regulation (EC) No. 1829/2003 the applicant did not present any data on the copy number of the transgenic insert in the genome of the potato. No such information has been made available to the national risk assessment bodies in the technical dossier, the respective annexes nor is there any reference concerning the copy number to be found in the final EFSA Opinions on GM potato EH92-527-1. Information about the insert copy number is requested by the respective EFSA guidance document, has to be included to the documentation, and has to be accessible for the national Competent Authorities. This information is an indispensable basis for a proper evaluation of the genotypic and phenotypic stability of the inserted trait and for the intensity of the intended effect (e.g. gene-dose effect).

Concerning the notification of GM potato EH92-527-1 according to Directive 2001/18/EC the applicant provides contradictory information:
He states that "Southern blot analysis indicated that one copy of the insert was integrated in potato clone EH92-527-1". On another occasion the notifier maintains that "two copies of the insert jointed tail to tail are integrated in amylopectin potato clone EH92-527-1…". However, data which could provide evidence for the assumed copy number are not presented ("data not shown") or cannot be evaluated quantitatively due to an inferior quality of the presented Southern blot photos (intense background staining and/or indiscriminative smears; inadequate experimental design to obtain quantitative results; see technical dossier Annex 4, Figures 5A and 5D).

Data presented in the Annex III update (Dossier acc. to Dir. 2001/18/EC) – Annex 3 pp93 cannot be evaluated properly because of the following reasons:

- high background staining of the Southern blots (Figures 3, 5, 8)
- target bands outside of the range of the molecular weight marker (Figure 6)
- superstaining of the positive control overlapping several additional lanes (Figure 6)
- smear in Prevalent DNA containing lane (Figure 8)
- tetraploid potato genome would produce overlapping fragments not detectable by this approach leading to a copy number bias.

As the potato genome is tetraploid and the insert locus lies within AT-rich inverted repeat genomic sequences the submitted data are not sufficient to exclude the presence of more than one/two copies of the transgenic insert in the genome of GM potato EH92-527-1. Omitting clear information concerning quantitative aspects of the application exacerbates the risk assessment of GM potato EH92-527-1 and increases the degree of uncertainty of the final EFSA Opinion.

4.7. The organisation of the inserted genetic material at the insertion site and methods used for the characterisation

The absence of unintended parts of the transformation vector is displayed in Annex 5 of the technical dossier (EFSA-GMO-NL-2009-14): The notifier did not establish the detection limit for unintended parts of the transformation vector. Moreover, the EH92-527-1 DNA isolation shows severe DNA degradation compared to the Prevalent template. He also did not explain why he did not use the respective part of pHoxwG vector as probe. The presented photo of the Southern blot is nearly black (Figure 2), identical fragments derived from the Prevalent and the EH92-527-1 DNA isolation reside on different positions on the gel (Figure 3). The pBIN19 derived probe shows an intense hybridisation signal in lanes containing no target DNA additionally invalidating the detected banding patterns in the lanes containing genomic DNA (Figure 3). This inaccurate and sloppy experimental design exacerbates the risk assessment of GM potato EH92-527-1 and increases the degree of uncertainty of the final EFSA Opinion.

4.8. Sub-cellular location(s) of insert(s) (nucleus, chloroplasts, mitochondria, or maintained in a non integrated form) and methods for its determination

The notifier is unable to provide sequence information of the genomic 3' flanking region. Only the sequence of the inverted 5' flanking region, which was the result of a substantial rearrangement of the insert involving potato genomic sequences at the insertion locus, is presented as 3' flanking region in the technical dossier. The genomic sequence downstream of the insertion locus is still not available. This exacerbates the risk assessment of GM potato EH92-527-1 and increases the degree of uncertainty of the final EFSA Opinion.

To provide evidence for the exclusion of a transgenic insert localization in the genome of chloroplasts the notifier presents data from Southern blots using extremely low amounts of chloroplast DNA as target (0,8 µg of chloroplast DNA versus 2 –10 µg/lane of target DNA as recommended by the manufacturer of the applied detection system (Amersham ECL Direct™ Nucleic Acid Labelling and Detection System)). Applying the manufacture's recommendation would have added substantially to the reliability of the presented conclusion.

4.9. Information on the expression of the insert

The notifier did not provide any quantitative data concerning the amount of antisense gbss mRNA produced by the transgenic insert. The introduced genetic modification (= antisense gbss RNA transcribed from the transgenic insert) is the test item. Quantitative data concerning the test item are requested and cannot be simply replaced by presenting indirect and at best semiquantitative data of a surrogate marker (= endogenously produced putative – but not identified by Western blot techniques - GBSS protein on a polyacrylamide gel).

The only data touching gbss expression of the transgenic insert is provided in Annex 8 of the technical dossier (EFSA-GMO-NL-2009-14; = Annex 14, C/SE/96/3501). However, evidence is indirect and the quality of the data (i.e. SDS PAGE photos) is inferior.

<u>The presented polyacrylamide gel in Annex 8 (Figure 3) suffers from the following flaws:</u>

- The putative GBSS band has not been verified via immunostaining on a Western blot.
- The molecular weight of the putative GBSS band cannot be determined unequivocally because the lane was overloaded and the respective band is represented by a smear ranging from approx. 56 to 76 kDa.
- The visible fragment pattern of EH92-527-1 and wild type potato flour differ at several other positions besides the putative GBSS band.
- The gel photo was cut off at approx. 30 kDa. Potentially present lower molecular weight bands are not displayed.

- The banding patterns of EH92-527-1 and wild type potato flour in Figure 1 besides the putative GBSS band are also obviously different.
- The quality of the SDS PAGE gel photos of both figures is inferior and does not allow an unequivocal interpretation of the results.
- The problems addressed by the notifier himself ("The lower band from potato flour, estimated to approximately 40 KDa, is **probably** retarded because of the huge amount of GBSS protein in the band above…") could have been easily resolved – but have not been done – by repeating the simple experiment with lower amounts of protein loaded.
- The notifier himself is not quite sure about the effect of the transgenic gbss antisense construct ("This is consistent with the **probable** silencing of the GBSS coding gene…"; "…we have **most probably** silenced the gene coding for the GBSS protein using antisense technique.").

In conclusion the notifier did not provide any data concerning the characterization and the amount of expression of the produced antisense gbss pre mRNA. Such information is crucial for the risk assessment of the application and required by the respective EFSA guidance document (EFSA 2006c). The notifier identifies ambiguities in the results himself but is not willing to solve them. Omitting this information exacerbates the risk assessment of GM potato EH92-527-1 and increases the degree of uncertainty of the final EFSA Opinion.

4.10. Genetic stability of the insert and phenotypic stability of the GM plant

The notifier provides evidence for the phenotypic stability of the intended trait by simply quantifying the amylose content of EH92-527-1 over several successive years. By doing so he refers to a wrongly labeled table which indicates an amylose content of EH92-527-1 of 26% compared to only 7,5 % amylose in the non-transformed potato variety Prevalent in the first year (see technical dossier, Table 2). Assuming that the notifier means that EH92-527-1 shows an amylose content of 7,5% the amylose content is decreasing constantly from 7,5 % to less than 2 % during the test period of 4 years. However, these data imply that the amylose content in the first year is 375 % higher compared to the content in the last year. Data showing such high variations in the concentration of the test item are inappropriate to provide evidence for genetic and phenotypic stability.

Considering data presented in Annex 25 of the technical dossier (EFSA-GMO-NL-2009-14), which is used by the notifier as proof of evidence for genetic and phenotypic stability of the trait, the following shortcomings became apparent:

1) unit descriptions in the chromatograms (x- and y-axis; see page 6) are missing
2) the overall visual quality of the graphs is extremely poor
3) there is no peak designation, the peak separation is poor

4) there is no quantitative evaluation of the peak areas (e.g. area under the curve) which is indispensable if the notifier wants to present percentages of amylose/amylopectin contents
5) the presentation of a positive amylose control is missing
6) the last line of Table 4 shows a wrong designation

Considering points 1 – 6 and the overall insensitivity of the procedure towards amylose the GPC method does not qualify to provide conclusive data concerning the quantity of amylose in EH92-527-1. This circumstance exacerbates the risk assessment of GM potato EH92-527-1 and increases the degree of uncertainty of the final EFSA Opinion.

4.11. Potential for gene transfer

The notifier presents data concerning the prevalence of kanamycin resistance in soil bacteria (see Appendix 26, technical dossier, EFSA-GMO-NL-2009-14). The sampling locations for the analyzed soils have not been identified impeding an evaluation of the relevance of the results concerning putative areas for cultivation of the transgenic potato. A classification of the soil is missing. The amount of soil used for analysis is not indicated impeding proper comparison with available literature data. The usually reported number of cultivable bacteria retrieved from 1 g of soil is lower than indicated by the notifier. The obtained number of resistant colonies is one order of magnitude higher than usually reported in the scientific literature. Neither colonies have been taxonomically identified nor has the genetic basis of the resistant phenotype been characterized. The presence of nptII genes has not been established nor has it been quantified.
These shortcomings and the naïve experimental design do not allow to draw any valid conclusions on the potential of GM potato EH92-527-1 to increase kanamycin resistance in soil bacteria.

5. Monitoring of cultivation of GM potato EH92-527-1

5.1. General remarks

Austria submitted reasoned objections against this notification on 25th June 2004 and again on 26th January 2005, with regard to concerns about the environmental risk assessment and monitoring. The objection was upheld taking into account the discussions and clarifications provided by the notifier on 11th January 2005. Several other Member States also upheld their objections concerning aspects related to monitoring of GM potato EH92-527-1 after discussions at a meeting on 11th January 2005.

On 2nd March 2010 Decisions were published by the European Commission to grant authorisation of the proposed use of GM potato EH92-527-1 according to the notifications under Directive 2001/18/EC and Regulation (EC) No 1829/2003 (It has to be remarked that the notification submitted according to Regulation (EC) No 1829/2003 in 2005 is also referring to the monitoring plan submitted in the framework of the notification according to Directive 2001/18/EC).
The Swedish Board of Agriculture has taken a decision on 31st March 2010 to place GM potato EH92-527-1 on the market for cultivation and industrial use, in compliance with the European Commission Decision under Directive 2001/18/EC (2010/135/EU) of 2nd March 2010.
The Swedish Board of Agriculture is currently discussing with the consent holder, BASF Plant Science how to implement the monitoring as laid out in the Annex of the decision of the European Commission. At the same time a decision to add GM potato EH92-527-1 to the Swedish National variety list was taken. On 10th April 2010 this product was registered in the Common Catalogue of Varieties of Agricultural Plant Science.
The COM Decision (2010/135/EU) of 2nd March 2010 in its Annex only provides a very general obligation to undertake field studies to monitor for potential adverse effects on potato-feeding organisms in the fields where GM potato EH92-527-1 is cultivated and reconfirms the proposed monitoring with a view to discussions between the consent holder and the Competent Authorities of the Member States.

5.2. Flaws in the risk assessment with relevance for monitoring

Austria issued concerns related to the notified changes in composition of GM potato EH92-527-1 according to point 2.5 of the environmental risk assessment (ERA): “A change in these parameters (note: mono- and disaccharides, vitamin C, glycoalkaloid level) could indicate that next to the insertion of the genes of interest other changes have occurred. They may be a direct consequence of the modification or result from epigenetic changes or somaclonal variation. These deviations may remain unnoticed and, if deleterious, could affect other organisms” (SBA 2004). As noted in the objection submitted by Austria on 25 June 2004 a detailed reflection of these aspects ERA is not contained in the monitoring plan, except for the requirement for CSM-monitoring

measures for effects of GM potato EH92-527-1 on organisms present in and around the cultivation fields according to the European Commission decision according to Directive 2001/18/EC.

Further concerns were expressed about the presence of the antibiotic marker gene nptII, with aspects relevant to monitoring (e.g. presence and persistence of intact nptII gene in the environment, likelihood of transfer of the nptII gene to bacteria taking into account the changed characteristics of GM potato EH92-527-1) (Wögerbauer 2007; BMGFJ 2007).

Additionally concerns have been raised with regard to uses of GM potato EH92-527-1 other than for industrial purposes, namely feed use of material produced from GM potato EH92-527-1 and the low level presence of GM potato EH92-527-1 in food and feed. It was criticised that only the monitoring plan according to Directive 2001/18/EC was attached to the notification under Regulation (EC) No 1829/2003 (Annex 43) and this plan does not adequately consider food and feed use of GM potato EH92-527-1.

In general uncertainties associated with the ERA of GM potato EH92-527-1 should be comprehensively addressed by monitoring according to a monitoring plan available at the time of consent. To address this issue the consent holder only issues a very general outline lacking relevant details how risk assessment assumptions are covered by case-specific monitoring or general surveillance (Table 1 of reply to Comments and Objections raised under Directive 2001/18/EC by the notifier as of 11 January 2005). The following chapter is describing the shortcomings of the currently available monitoring plan for GM potato EH92-527-1.

5.3. Shortcomings of the proposed monitoring plan

5.3.1. General shortcomings

As criticised earlier (in objections against the notification C/SE/96/3501 and in the comments of the Austrian competent authority on the notification EFSA/GMO/UK/2005/14 under Regulation (EC) No 1829/2003) the monitoring plan provided by the notifier is not deemed comprehensive enough and is not elaborated in adequate detail.

The revisions need to address the details of certain procedures for the monitoring which are outlined in the current monitoring plan but lack necessary elements: reference to standardised methods, protocols of methods for collection of data, scope of investigations, methods for analysing data which are in line with the state of the art. Thus a fully specified list of monitoring parameters needs to be established in relation to the potential environmental effects of GM potato EH92-527-1, together with detailed information according to each parameter as regards monitoring methods, timing and locations for monitoring.

In this respect the recommendations for monitoring issues, methods and location & timing of monitoring measures as outlined in the comprehensive monitoring checklist for GM potatoes with altered starch content (MWG 2008c) established by the Monitoring working group of the Commission and the Competent Authorities under Directive 2001/18/EC (MWG 2008a) should be fully considered to conceptionally implement the legal framework for monitoring (Züghart et al. 2008).
Additionally the representativeness of sampling with regard to large-scale application of GM potato EH92-527-1 needs to be explained further than in the current monitoring plan, taking into account general considerations for choosing representative trial locations (see e.g. EFSA 2006c and 2010a).

Secondly the revision of the monitoring plan needs to address all potential routes of environmental exposition for cultivation and import of GM potato EH92-527-1. E.g., if whole GM potatoes are being imported into Austria the risk of accidental spillage or release of tubers during transport and handling should be considered. In this respect the survival of viable transgenic potato tubers over winters cannot be excluded and should be subject to monitoring: As described by Askew (1991) in Bond et al. (2007), between 370.000 and 460.000 tubers per ha remain in the soil after potato harvest. Consequently more volunteer plants than plants growing from commercial seed can be expected (British Potato Council (2005). Climate changes caused by global warming already influence the potato production. Extremely hot and dry weather during summer often causes physiological alterations such as re-growth and secondary tuber formation which result in a high number of small tubers that are left in the soil after harvest. If they do not freeze during the following winter, they can cause serious problems in the following crop or even later in rotation. In Slovenia for example most of the tubers survived the winter 2006/2007. In the future even bigger problems with volunteer growth are expected (Dolnicar et al. 2008). A (chemical) treatment of volunteers in the following crop is difficult and the development of new tubers may cause additional problems during the next planting season (Schächtl 2007; www.proplanta.de). Moreover these volunteers cause also problems during crop rotation, especially in poorly competitive crops such as leeks and onions as well as peas, beans and carrots for processing grown in the next three years after potato cultivation (Orson (1994) in Bond et al. 2007).
If monitoring fails to identify volunteer plants in the subsequent seasons, then the potato volunteers reduce yield, hinder harvesting and contaminate the produce (Bond et al. 2007) – in case of GM potato EH92-527-1 with a genetically modified plant, that is not intended for human consumption!

Furthermore the revised monitoring plan needs to confirm that reports will contain all established data not only summaries of results and the necessary information to assess whether adequate methods were used for analysis of the data (e.g. as described in EFSA 2010b).

Until the above mentioned revisions to the current monitoring plan are not submitted and assessed for adequacy, potential application of GM potato EH92-527-1 in Austria cannot be comprehensively monitored and risk assessment assumptions thus cannot be validated in the necessary detail.
Furthermore any relevant lessons derived from experiences with the monitoring of cultivation and use of other GM crops (e.g. GM maize MON810) should be taken into account for revision of the current monitoring plan for GM potato EH92-527-1.

5.3.2 Flaws in the Case-Specific Monitoring (CSM)

The current CSM plan needs to be revised to specifically integrate the obligation to undertake field studies to monitor for potential adverse effects on potato-feeding organisms in the fields where GM potato EH92-527-1 is cultivated. In this respect it needs also to address the fact that the "Increased content of sugar and reduced content of glycoalkaloids might possibly result in pests (e.g. insects) attacking the potato to a larger extent", as outlined in the risk assessment report by the Swedish authority (SBA 2004).

The monitoring therefore needs to specifically address whether the substantial differences identified for these compounds (highly significant increase of mono- and disaccharide content and decrease of solanin and chaconin content) have consequences for the susceptibility of the GM potato cultures to all relevant potato pests and diseases (e.g. Oehmichen 1986). The monitoring needs to take into consideration any single or combined effects of these compositional changes since the different pest species react differently to increased sugar and/or decreased glycoalkaloid contents. Established monitoring systems for potato pests and diseases need to be integrated into the monitoring (Grünbacher et al. 2007). The indirect effects of any changes in susceptibility to pests need to be taken in consideration as well, since consequently a higher amount of pesticides may be needed for cultivation management. Therefore the indirect effects caused by any additional application of pesticides on the environment should be addressed by the monitoring as well.

Further the improved CSM plan needs to clarify, that the proposed monitoring will encompass the investigation of effects of the GM potato EH92-527-1 on the microorganism-flora of the soil. The monitoring needs also to address any impacts on decomposition processes in the soil. Based on his risk assessment the consent holder argued that such effects would not be likely, but this assumption needs to be substantiated.
In this context it has to be remarked that the possibility of horizontal gene transfer of antibiotic resistance marker genes to soil microorganisms should be taken into account.

The monitoring needs also to address how GM potato EH92-527-1 differs from the recipient plant in reproduction, dissemination as well as survivability in a scientifically sound manner in addition to the general agronomic and selected compositional

parameters included in the current monitoring plan. Specifically the assumed rate of flower abortion for GM potato EH92-527-1 needs to be investigated with adequate methods. This assumption further needs to be confirmed under the conditions of larger-scale applications as during cultivation for industrial purposes.
The monitoring also needs to address any further differences, which may accompany the identified compositional changes in GM potato EH92-527-1 as noted in the above mentioned assessment report, but which were not directly assessed during the evaluation for risk assessment (i.e. changes other than the investigated standard compositional parameters and agronomic characteristics).
A specific point in question which is not covered by the current monitoring is the issue whether the modified starch content and the increase in mono- and disaccharide content is influencing frost tolerance of the tubers of GM potato EH92-527-1. Currently the consent holder assumes that this can be addressed by indirectly monitoring of GM potato volunteers according to the proposed plan without further revisions. However the issue needs also to be addressed directly to provide meaningful results.

The concept of volunteer monitoring presented in the monitoring plan assumes that potatoes are not planted in consecutive years due to crop rotation schemes and volunteer plants could be detected in the year following cultivation of GM potato EH92-527-1 and managed by the notified methods. However with a view to the possible occurrence of several mild winters following each other, as happened in Austria in the years before 2008/2009, longer dormancy periods need to be considered as well. Any monitoring therefore needs to specifically assess the following factors in order to establish data to estimate the potential for contamination of potato-products produced for the food- and feed-supply:

- winter temperatures and snow covering periods in the cultivation areas,
- relevant crop rotation factors, specifically with respect to subsequent potato cultivation,
- cultivated potato varieties, specifically the use of nematode resistant potato-varieties which allow shorter rotation schemes for potato cultivation,
- methods of harvest and tillage procedures,
- management of subsequent crops.

With widespread cultivation of GM potato EH92-527-1 only a comprehensive monitoring system which is substantially improved compared to the proposed monitoring plan will be able to identify the establishment of volunteers, which could eventually result in admixtures with following non-GM potato cultures that should be prevented and which would not be detected by the proposed identity preservation system.

5.3.3 General surveillance (GS) of unforeseen and long-term effects

The general surveillance plan proposed by the notifier is too general and unspecific to allow the detection of potential adverse effects upon of GM potato EH92-527-1, specifically of unanticipated adverse effects. Thus the current plan does not meet the requirements set out in Annex VII of Directive 2001/18/EC.

The current GS plan does not address the spread persistence and potential accumulation in the soil of the transgenic sequences present in GM potato EH92-527-1, which is considered an important element of monitoring (Züghart W., and A. Doerpinghaus 2004). It also does not utilise scientific methods for the investigation of effects of biodiversity, except the organisms investigated during the CSM. Instead the proposed GS focuses on feedback from the growers, which have an obligation to respond to the GS questionnaires under the specific requirements for cultivation of GM potato EH92-527-1 (production contracts).

In addition to potato growers and industrial processors of starch potatoes the proposed monitoring plan describes the implication of other key existing networks, among them non-agronomical experts for the surveillance of the receiving environments and human and veterinary health institutions.
Currently it is not clear how unanticipated effects on human and animal health can be assessed by the proposed monitoring plan. Furthermore it remains unclear, how the relevant networks will be included in the monitoring activities, and which methods will be used in order to detect relevant possible adverse effects. However a detailed monitoring plan addressing such questions is considered necessary specifically considering the fact that GM potato EH92-527-1 is the first application of this kind (i.e. starch content altered potato) and raised concerns with regard to health issues (e.g. concerns with regard to the nptII-transgene contained in GM potato EH92-527-1). In view of the fact that GM potato EH92-527-1 material will be used as animal feed, a robust monitoring plan in order to ensure safety of this product for animal health is particularly important.

The experience with monitoring of GM maize MON810 in Germany showed that it is instrumental to have an agreeable monitoring plan in place which is detailing the necessary measures to fulfil the obligations required by the consent before the monitoring is conducted (Gathmann 2008, Monsanto 2009, Vogel 2009). Without a final and agreed monitoring plan at hands the authorities cannot assure that the monitoring is conducted sufficiently and the established results adequately address the objectives of the monitoring (COM 2010/135/EU, Article 4, Para 1b – 1d).

The identity preservation (IP) system according to COM decision (2010/135/EU) Article 4, Para 1a is considered as an important tool in the proposed monitoring system, however it can only detect unexpected incidences (e.g. like the presence of admixtures between GM potato EH92-527-1 and other potatoes with relevance for the quality of

the product) on the foreseen routes of production, i.e. the growers and the industrial processors. GM potato EH92-527-1 material, which is unexpectedly introduced into other production channels, e.g. into food- and feed-production by second year volunteer plants or accidental spillage and mixing with other sources would not necessarily be detected by the proposed IP-system.

In summary details on networks involved in GS, their expertise with regard to the issues for monitoring, the methodology used for monitoring, location and frequency of monitoring activities must be provided before placing on the market of GM potato EH92-527-1 in Austria.

5.3.4. Conclusion

The currently available monitoring plan for GM potato EH92-527-1 is not sufficient to meet either the general requirements of Annex VII of Directive 2001/18/EC or the obligations according to COM decision 2010/135/EU.

The monitoring plan has therefore to be significantly improved in the following ways:

- in general terms as outlined in chapter 2.1,
- with regard to Case-Specific Monitoring (see chapter 2.2) taking into account methodological aspects of the recently proposed guidance for ERA (EFSA 2010a) and
- with regard to General Surveillance (see Chapter 2.3) taking into account the recent experiences with monitoring for cultivation of GM crops in Europe (Gathmann 2008, Monsanto 2009, Vogel 2009) and recommendations of the Monitoring Working Group (MWG 2008b).

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The ANNEX containing information/figures on the quality of provided Southern blots and silencing of endogenous gbss expression is considered as confidential business information and only provided for communication with EC, EFSA and Competent Authorities according to Directive 2001/18/EC and Regulation (EC) No. 1829/2003.