



## WISSENSCHAFTLICHE BEGRÜNDUNG FÜR EIN IMPORTVERBOT VON GENETISCH VERÄNDERTEM MAIS MON 863 (*Zea mays* L., Linie MON 863) DER FIRMA MONSANTO (NOTIFICATION C/DE/02/9)

### ZUSAMMENFASSUNG

Die Zulassung von genetisch verändertem Mais MON 863 erfolgte als Entscheidung der Kommission (2005/608/EG) gemäß Richtlinie 2001/18/EG mit 8. August 2005. Das Produkt kann wie sonstiger Mais verwendet werden, ausgenommen für Anbauzwecke und die Verwendung als oder in Lebensmittel(n). Mit 13. Januar 2006 erfolgte die Genehmigung des Inverkehrbringens von aus der genetisch veränderten Maissorte MON 863 gewonnenen Lebensmitteln und Lebensmittelzutaten als neuartige Lebensmittel oder neuartige Lebensmittelzutaten gemäß der Verordnung (EG) Nr. 258/97 des Europäischen Parlaments und des Rates.

Hinsichtlich der toxikologischen **Sicherheitsbewertung** wurden zahlreiche Mängel festgestellt:

Hinsichtlich jener **Fütterungsstudien**, welche in HAMMOND et al. (2006) als wissenschaftlicher Beweis für die Sicherheit von Mais MON 863 zitiert werden, sind folgende Defizite evident:

- 1) Hinsichtlich des Versuchsdesigns ist kritisch anzumerken, dass die Referenzgruppen 60-80% der Maisprobe darstellen. Statistisch signifikante Unterschiede zwischen den Test- und Kontrollgruppen bleiben dadurch verborgen.
- 2) Das verwendete Futtermittel wurde in den meisten Fällen nicht auf das Vorhandensein von GVO-Verunreinigungen getestet.
- 3) Eine klare Dokumentation über die Futterzubereitung (z.B. thermisches Prozessieren) fehlt.
- 4) Studien zum Vitamingehalt der Futtermittel fehlen.
- 5) Hygienische Standards wurden zum Teil nur sehr ungenau beschrieben.

Aus diesen Gründen sind die Ergebnisse dieser Studien zu hinterfragen und sind somit nicht geeignet, die Unbedenklichkeit des Produkts zu belegen. Darüber hinaus handelt es sich bei diesen Arbeiten um keine Studien zur Beurteilung einer allfälligen chronischen Toxizität des Produkts.

Hinsichtlich der Studie von Monsanto (inkl. HAMMOND-Artikel) zur **toxikologischen Prüfung** sind weitere schwere Mängel ersichtlich:

- 1) Eine veraltete Version der OECD-Guideline No. 408 wurde verwendet, obwohl die aktuelle Version schon zum Zeitpunkt des Versuchsbeginns von Monsanto zwei Jahre in Kraft war. Dadurch sind im Studiendesign zahlreiche Abweichungen klar erkennbar, die nicht als dem Stand von Wissenschaft und Technik entsprechend zu werten sind.
- 2) Hinsichtlich des verwendeten Futters ist festzuhalten, dass weder Informationen zur Futtermittelzubereitung noch über die Einhaltung hygienischer Standards sowie der Art der Tierhaltung hinreichend gegeben sind.
- 3) Es fehlen die Ergebnisse der mikroskopischen Untersuchung des Knochmarks der Testorganismen, obwohl es beprobt wurde.
- 4) Gewichtsanalysen von Thymus und Uterus fehlen gänzlich.
- 5) Studiendesign und gewählte statistische Methode sind dahingehend zu hinterfragen, welches Ausmaß an Unterschieden damit erkennbar ist bzw. ob damit nicht nur große Abweichungen von der Kontrollgruppe erkennbar sind.

Bezüglich der im Anschluss durchgeführten Reevaluierung der Daten kann festgehalten werden, dass diese auf Grund der oben genannten schweren Mängel, die bei deren Generierung offensichtlich vorlagen, nicht nach dem aktuellen Stand von Wissenschaft und Technik entsprechend gewonnen wurden und somit zur Untermauerung der Sicherheit von Mais MON 863 nicht geeignet sind.

Folgende **Empfehlungen** sollten daher umgesetzt werden:

- 1) Auf Grund aller in dieser Stellungnahme angeführten Mängel, ist eine komplette Wiederholung der ursprünglichen Monsanto-Studie nach dem aktuellen Stand von Wissenschaft und Technik durch unabhängige Wissenschaftler notwendig.
- 2) Einschlägige toxikologische Untersuchungen von Mais MON 863 sollen Studiendesigns berücksichtigen, welche sensitive Perioden - wie Reproduktion und Alterung - umfassen.
- 3) Die Risikoforschung, durchgeführt von unabhängigen Institutionen, soll intensiviert werden.

Hinsichtlich der **Sicherheitsbewertung des Antibiotikaresistenzmarkergens (ARM-Gen) nptII**, wurden ebenfalls zahlreiche Mängel festgestellt:

1) Die Darstellung im Antrag, dass die meisten Antibiotika, welche durch diese Resistenzgene inaktiviert werden, keine **therapeutische Relevanz** in der Human- und Tiermedizin aufweisen und nicht mehr länger verwendet werden, ist nicht korrekt. In Stellungnahmen der WHO, welche Kanamycin und Neomycin in die Gruppe der „critically important antibiotics“ reiht und der Europäischen Arzneimittelagentur (EMA) wird eindeutig die Wichtigkeit dieser Antibiotika in der Veterinär –und Humanmedizin belegt.

Auch die These, dass die natürliche Resistenzsituation gegenüber Kanamycin und anderen Aminoglykosiden in allen Ländern gleich sei, kann wissenschaftlich nicht aufrecht erhalten werden. Länderspezifische Unterschiede in der Art und Häufigkeit der Anwendung dieser Antibiotika sowie im Vorkommen von natürlichen Resistenzen sind in die Risikobewertung einzubeziehen.

2) Weiters ist eine niedrige Transferhäufigkeit von ARM Genen in natürlichen Habitaten nicht mit einem niedrigen Risiko für negative Auswirkungen auf Gesundheit und Umwelt gleichzusetzen: **Transferfrequenzen** besitzen wenig prädiktive Aussagekraft über Langzeitfolgen von sporadisch vorkommenden Gentransfervorgängen. Dieser Umstand ist gegenständlich besonders prekär, da es berechnete Zweifel gibt, ob mit den zurzeit zur Verfügung stehenden Mitteln und Methoden diese seltenen – aber sehr wohl relevanten - Transfervorgänge überhaupt erfasst werden können. Überdies kann eine einzige erfolgreiche ARM Gen Übertragung ausreichen, um einen in weiterer Folge resistenten Stamm zu bilden.

3) Substantiell unterschiedliche länderspezifische Anwendungs- und Verbrauchsmuster von Antibiotikapräparaten müssen Berücksichtigung in der Sicherheitsbewertung finden und dürfen nicht übergangen werden, da ansonsten die im natürlichen Habitat herrschenden **Selektionsdrücke** nicht richtig eingeschätzt werden können. Der dynamische Charakter der Hintergrundbelastung mit Resistenzgenen darf nicht ignoriert werden, da diese Resistenzfunktionen via horizontalen Gentransfer leicht ausgetauscht werden und sich Bakterienpopulationen so rasch an sich verändernde Umweltbedingungen anpassen können. Dies beinhaltet auch einen eventuellen Rückgang der entsprechenden Resistenzraten.

4) Es wird undifferenziert von einer hohen **Hintergrundbelastung** mit Resistenzdeterminanten in natürlich vorkommenden Bakterienpopulationen ausgegangen. Dieser Standpunkt vermittelt den Eindruck, dass die zusätzliche Einbringung von Resistenzfunktionen via ARM Gene in den natürlichen Resistenzgenpool ohne wesentliche Auswirkungen bleibt. Aber die Annahme einer hohen Hintergrundbelastung mit Resistenzgenen zieht weder stamm- oder speziesspezifische Unterschiede in den Resistenzraten in Betracht, noch beachtet sie völlig unterschiedliche Resistenzraten bei ein und der selben Spezies in unterschiedlichen Ländern.

5) Die Erfassung von resistenten Stämmen in potentiellen Rezeptorpopulationen (=Hintergrundbelastung mit Resistenzen) hat **quantitative Angaben der potentiell über GMOs eingebrachten ARM Gene** (=Kopienanzahl) zu beinhalten. Ein quantitatives Verständnis dieses Phänomens ist unabdingbar, um die Auswirkungen zusätzlich eingebrachter ARM Gene tatsächlich und ernsthaft abschätzen zu können.

6) Die Annahme, dass in natürlichen Habitaten (Felder, Boden etc.) kein oder kaum ausreichend großer **Selektionsdruck** herrscht, um raren ARM Gen Transformanten einen Wachstumsvorteil zu ermöglichen, der ihnen erlaubt, einen relevanten Anteil in der Bakterienpopulation einzunehmen, wird durch jüngste Daten in Frage gestellt. Gerade im Ackerboden, auf und in Pflanzen sind bedeutende Mengen an Antibiotika anzutreffen, die auch über längere Zeit persistieren und durchaus in der Lage sind, positiven Selektionsdruck auszuüben. Die Anwendung von Antibiotika im veterinärmedizinischen Bereich erzeugt ebenfalls beachtlichen Selektionsdruck in Bakterienpopulationen.

In Übereinstimmung mit der ad hoc Gruppe des Norwegian Scientific Panel on Genetically Modified Organisms and the Panel of Biohazards wurden signifikante und deutliche Unterschiede zwischen den Mengen des Antibiotikaverbrauchs und

den Antibiotikaresistenzraten in den europäischen Staaten festgestellt. Somit ist die Anwendung des „**Fall zu Fall-Prinzips**“ unter Berücksichtigung der länderspezifischen Besonderheiten unumgänglich.

Folgende **Empfehlungen** sollten daher umgesetzt werden:

- 1) Auf Grund der fehlenden quantitativen Daten hinsichtlich des nptII-Gens und seiner Freisetzung, bzw. Marktzulassung, soll die Risikoforschung durch unabhängige Institutionen verstärkt werden.
- 2) Zum Monitoring der Hintergrundbelastung mit Antibiotikaresistenzgenen sollen geeignete bakterielle Referenzstämme ausgewählt werden.
- 3) Vor der Markteinführung von gentechnisch veränderten Produkten, welche ARM-Gene enthalten, ist eine besondere Vorsicht in Ländern mit einer geringen Inzidenz für aminoglykosidresistente pathogene Keime geboten. Eine massive Verbreitung von DNA-Fragmenten, welche das nptII-Gen enthalten, über die transgene Pflanze wird mit Sicherheit zu Veränderungen in der örtlichen Exposition und der Expositionsrate bei Bodenbakterien und jenen des Magen-Darm-Trakts führen.
- 4) Auf Grund der zeitlichen und örtlichen Beschränkung bei Freisetzungsversuchen, könnte auch die Gelegenheit für einen erfolgreichen Gentransfer von GVOs auf Bakterien limitiert sein. Dies trifft aber nicht zu, wenn transgene Produkte kommerzialisiert und somit Bakterien über Jahrzehnte großflächig ARM-Genen ausgesetzt werden.
- 5) All diese Fakten unterstreichen die österreichische Position, dass eine umfassende Risikobewertung des nptII-Gens, welche eine Evaluierung von potenziellen indirekten und Langzeiteffekten beinhaltet, durchgeführt werden muss.

Hinsichtlich der Problematik zur **unbeabsichtigten Ausbringung** von Mais MON 863 und der entsprechenden Maßnahmen zum **Risikomanagement** kann folgendes festgehalten werden:

- 1) Gemäß Annex VII der Richtlinie 2001/18/EG hat dem Dossier ein Monitoringplan, welcher auch die Überwachung von möglichen unvorhergesehenen negativen Effekten umfassen soll, angeschlossen zu sein.
- 2) Die unbeabsichtigte Ausbringung erfolgt beispielsweise durch Transportverluste und stellt somit eine unbeabsichtigte Freisetzung von GVO in die Umwelt dar. Zusätzlich kann eine Umweltexposition Bt-Toxins beispielsweise über die Ausbringung von Stallmist bei der Verwendung als Futtermittel erfolgen.
- 3) Der derzeitige Monitoringplan sieht keine Überwachung im Hinblick auf die unbeabsichtigte Ausbringung vor, wodurch mögliche negative ökologische Auswirkungen auf die Umwelt nicht erfasst werden.

Folgende **Empfehlung** sollte daher umgesetzt werden:

Der vorgeschlagene Monitoringplan entspricht nicht den Vorgaben des Annex VII der Richtlinie 2001/18/EG sowie der Entscheidung des Rates 2002/811/EG. Somit ist als angebracht anzusehen, dass ein adäquates Update zum Monitoringplan implementiert wird.

Österreich hatte sich deshalb - gemeinsam mit einer Reihe von anderen Mitgliedstaaten - bereits im Rahmen des Zulassungsverfahrens nach der Freisetzungsrichtlinie wiederholt gegen das Inverkehrbringen dieses Produkts ausgesprochen. Im Regelungsausschuss vom 29. November 2004 sowie im Umweltrat vom 24. Juni 2005 stimmte in der Folge eine große Mehrheit (142 Stimmen) gegen die Marktzulassung. Trotzdem hat die EK mit 8. August 2005 dieses Produkt EU-weit zugelassen hat. Österreich vertritt aber nach wie vor die Meinung, dass die Risiken dieses Produkts hinsichtlich der Gesundheit von Mensch und Tier sowie der Umwelt nicht adäquat durch den Antragsteller bewertet worden sind. Diese wissenschaftliche Argumentation wird auch anhand neuer bzw. zusätzlicher wissenschaftlicher Erkenntnisse gestützt. In der Folge hat Österreich ein befristetes Importverbot bis 1. Oktober 2010 erlassen. Dieser Zeitraum soll auch zum wissenschaftlichen Diskurs über dieses Produkt genutzt werden.

## SCIENTIFIC ARGUMENTS FOR AN IMPORT BAN OF GENETICALLY MODIFIED MAIZE MON 863 (*Zea mays* L., line MON 863) OF MONSANTO (NOTIFICATION C/DE/02/9)

### SUMMARY

On 8th August 2005 the Decision (2005/608/EC) concerning the placing on the market, in accordance with Directive 2001/18/EC of genetically modified maize MON 863 was adopted by the Commission. The product may be placed on the market and put to the same uses as any other maize, with the exception of cultivation and uses as or in food.

On 13th January 2006 the placing on the market of foods and food ingredients derived from genetically modified maize line MON 863 as novel foods or novel food ingredients under Regulation (EC) No 258/97 was authorised.

Austria had – in line with a lot of other member states – voted against the placing on the market of this product during the Regulatory Committee acc. to Directive 2001/18/EC on 29<sup>th</sup> November 2004 and the Environmental Council on 24<sup>th</sup> June 2005, where a representative - though not qualified - majority could be reached (142 votes against the placing on the market). Nevertheless, the EC decided on 8<sup>th</sup> August 2005 in favour of this product, which got approval for placing on the market.

In this document, the scientific arguments, which are justifying the Austrian import ban of this GMO, are described. They focus particularly on the toxicological safety assessment and the antibiotic resistance marker (ARM) gene *nptII*, which is contained in maize MON 863, but also on the given risk management measures to prevent accidental spillage.

**Summarizing** the evaluation of the **toxicological safety assessment** of the dossier, it can be stated that a lot of deficiencies are obvious:

With regard to the **studies on nutritional equivalence assessment in farm animals**, which are quoted in HAMMOND et al. (2006) as scientific proof for the safety of maize MON 863, a lot of shortcomings have been detected:

- a) Concerning the experimental design it has to be criticised that reference groups are often contributing 60-80% of the sample size. Statistically significant differences between test and control groups are therefore often masked because group differences between iso- and transgenic diets fall into the broad range of reference groups. Emphasis should be laid on the outcome of tests with closely related control groups.
- b) With regard to diets used, it has to be critically noted that the majority of the studies did neither investigate nor quantify the presence of GMO-contamination in the corn, nor in the diet that was fed to the animals.
- c) Further more a clear definition of the conditioning process is missing, which makes it impossible to evaluate the potential impact on DNA and proteins. Thus investigations of the modification should be done in the corn and in the end product, allowing complete assessment of the complete experimental diet.
- d) No study investigated vitamin contents of grain or the complete diet.

- e) Hygienic evaluation was very limited and included only mycotoxin analysis while other microbial traits were not measured. Only one study verified levels of pesticides.

However, results are strongly associated with the methods used and the traits investigated. Therefore the results of these studies have to be questioned and cannot be regarded as a scientific proof for the safety of maize MON 863. Additionally these studies cannot be used for statements or citations on chronic toxicity.

With regard to the **studies on toxicity assessment in laboratory animals** it has to be stated that also there many deficiencies are obvious:

- a) An outdated version of the OECD Guideline No 408 was used, omitting parameters that were already state of the art two years in advance of the beginning of the Monsanto study. Therefore a lot of deviations in the study design are obvious, which cannot be regarded as appropriate.
- b) Concerning the description of the diets used, it has to be remarked that the reference varieties were tested with different methods, which were not clearly defined: The authors did not mention if the rodent diets were pelleted or otherwise thermally treated or not.
- c) Additionally microbial counts, which are considered as important hygienic traits, were neither investigated in the grain nor in the diets.
- d) Further more there was no report on the microscopic examination of the bone marrow, though it was collected. The investigation of the bone marrow could have been supplementary to the haematology showing differences between the groups.
- e) Thymus and uterus weight evaluations were completely missing. This can not be regarded as appropriate.
- f) An important factor is also the sensitivity of the animal model: HAMMOND et al. (2006) described the use of an outbred rat model. The study compared a high number of different lines of maize, among them MON 863. The data vary considerably in and between the groups. That would allow the assumption that only effects with great deviations from the control would have been detectable with the chosen trial setup.

With regard to supplementary considerations to HAMMOND et al. (2006) based on the **original Monsanto report** it has to be mentioned, that the data are not presented in a user friendly way: The report consists of an unclear collation of the various contributions. Also many deviations of the protocol give the study a difficult appearance.

- a) Again the use of an outdated version of the OECD Guideline No. 408 has to be criticised.
- b) Further more animal husbandry is insufficiently described.
- c) Little information is available on diet pre-treatment and feed technology used. As in the Hammond-paper, the question remains open whether the diets were pelleted or otherwise heat treated.
- d) GRANT et al. (2003) and TAYLOR et al. (2003) are cited to support the absence of unintended changes of MON 863 on animal health. This conclusion can again not be regarded as appropriate due to the many

deficiencies mentioned above and additionally lacks justification, as these studies do not represent strictly speaking "toxicity" studies.

- e) The statistical methods used in the Monsanto report can not be regarded as suitable for detecting differences in the response of the rats to the diet compared to the control diet.

Concerning the **re-analysis of data** it has to be mentioned that the EFSA analysis did not find any consistent pattern over dose and gender rendering changes of biological importance. But these findings are based on data, which were generated in a not state of the art way. Also all deficiencies identified and concluded in this scientific statement have to be taken into consideration. Therefore these data cannot be regarded as a suitable scientific proof for the human and animal safety of maize MON 863.

Therefore the following **recommendations** should be implemented:

- a) Due to the above mentioned deficiencies, a complete repetition of the Monsanto report by independent scientists is highly recommended.
- b) Investigations with maize MON 863 should include elaborate designs including sensitive periods as reproduction or ageing.
- c) Research by independent scientists and research groups should be supported in the future.

With regard to the **safety assessment of the ARM gene nptII**, also a lot of deficiencies have been detected:

1) The statement that most of the antibiotics inactivated by ARM genes are outdated and no longer in use does not take into account the actual **therapeutic relevance** of kanamycin, neomycin and paromomycin in human and veterinary medicine as well as the remarkable differences in antibiotic consumption and prescription patterns between different countries of the European Union. This diverging antibiotic usage leads to completely different resistance profiles of clinical isolates in each of these countries. These local peculiarities for the application of antimicrobials have to be taken into consideration for a risk assessment of the ARM gene under scrutiny.

Furthermore aminoglycosides as a class have become more and more important as alternative treatment options due to the rise of multi-resistant bacterial strains (e.g. *M. tuberculosis*). Kanamycin and neomycin have been classified as critically important antibiotic by WHO (2005).

2) The most prominent argument for an unrestricted use of ARM genes in transgenic plants is the marginal low **frequency of gene transfer** of ARM genes from the plant genome to competent bacteria in natural environments. EFSA argued that a low gene transfer frequency in natural habitats is equivalent to a low risk for adverse effects. Unfortunately, frequencies are of little predictive value in the assessment of long-term effects of sporadic gene transfer events, particularly because the relevant transfer frequencies may well be below current methodological detection thresholds. A single successful ARM gene uptake event may be sufficient to build a founder generation for a subsequently resistant bacterial strain.

3) With regard to the kanamycin and neomycin **resistance in natural environments** it has to be stated that the components of the global pool of resistance genes are in a constant flow, reacting to continually changing environmental conditions. Genetic information is constantly exchanged between the participants of the microbial ecosystem. Substantially different country-specific application patterns for antimicrobials are not taken into consideration and additionally the analysis does not consider the highly dynamic nature of resistance in natural environments, where resistance genes are readily exchanged via horizontal gene transfer adapting to the relevant selection pressure prevailing in the habitat. A generalizing statement about the status of resistance in natural environments is unreliable.

4) Indiscriminative high **background resistance levels** in naturally occurring bacterial populations are postulated: This statement communicates the impression of a low risk process if ARM genes additionally are introduced into an ecosystem. But this proposition does neither take into account strain- and species-specific differences in resistance levels, nor does it acknowledge locus-, habitat- and country-specific differences in the resistance rates of the same bacterial species. The actually occurring resistance status was verified with kanamycin and neomycin (nptII). This example provides evidence for a low prevalence of both resistance functions in many environments and strains analyzed.

5) In the risk assessment any quantitative data concerning the **copy number of resistance determinants in receptor populations** (background-level of resistance) or the potential input copy number of ARM genes via transgenic organisms is missing. But a quantitative understanding of this phenomenon is necessary for a serious assessment of the effect of additionally introduced ARM genes.

6) Recent data have indicated the presence of considerable amounts and **persistence** of various antibiotics in soil and on, and even in, plants from manure of animal husbandry. The application of antibiotics for the treatment of infectious diseases in animals also provides substantial stress on bacterial populations. Therefore positive selection pressure even in field environments is not unlikely.

In accordance with the ad hoc group of the Norwegian Scientific Panel on Genetically Modified Organisms and the Panel of Biohazards, significant and distinct differences in antibiotic usage levels and antibacterial resistance rates between European countries could be identified. This observation implies the necessity of a **case-by-case risk evaluation** of each notification taking into account country-specific peculiarities. A large-scale introduction of ARM genes via transgenic crop plants leads to a different outcome of the risk assessment in areas with a low incidence of the equivalent resistance functions compared to environments with an intrinsic high background level of resistance. This consideration is also relevant for the group 1 ARM gene nptII.

Therefore the following **recommendations** should be implemented:

1) Due to the lack of available convincing, quantitative data concerning the nptII gene and its deliberate release, respectively the placing on the market, risk research done by independent scientists should be carried out.

2) For monitoring of antibiotic resistance gene background load certain bacterial reference strains should be chosen.

3) Before introducing genetically modified products containing ARM-genes, special care should be taken in countries with a low incidence of aminoglycoside resistant pathogens.

A massive dissemination of nptII containing DNA fragments via transgenic crop plants will certainly lead to alterations in the exposure locus and exposure rate of soil and gut bacteria not previously available for these bacteria.

4) Due to the temporal and local restrictions of field experiments the opportunity for efficient gene transfer from GMOs to bacteria may be limited, but a contrary situation is given if transgenic crops are commercialized, exposing the bacterial communities for decades and over large areas to ARM-genes.

Therefore it is highly recommended - and also laid down in Directive 2001/18/EC 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 ARMs in GMOs which may have adverse effects on human health and the environment". As a consequence a reduction of the artificial ARM gene exposure level to bacterial communities to a minimum should be made.

5) All these scientific facts underline the Austrian position that a comprehensive risk assessment of the nptII gene including the evaluation of potential long-term and indirect effects has to be carried out before the placing on the market of products containing this ARM-gene. Therefore a reanalysis of the safety assessment of the nptII gene taking into account the above mentioned criteria by independent scientists is highly recommended.

With regard to the possibility of **accidental spillage** and the appropriate **risk management measures**, the following points have to be considered:

1) According to Annex VII of Directive 2001/18/EC a monitoring plan, which should cover surveillance for unanticipated adverse effects must be included. The objectives of the monitoring plan are to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the environmental risk assessment.

2) Accidental spillage presents one way of unintended release of maize MON 863 into the environment. In addition, exposure of the environment to the Bt toxin expressed in MON 863 (Cry3Bb1) is possible via the consumption and excretion of maize MON 863 when used as animal feed and has to be addressed in the dossier in an appropriate way.

3) The monitoring plan submitted for maize MON 863 does not foresee monitoring of accidental spillage and possible ecological consequences arising from accidental spillage or other forms of introduction of the transgene products in the environment. In addition, no specific risk management strategies in case of unintentional release are provided.

Therefore the following **recommendation** should be implemented:

The applicant's proposal for an environmental monitoring plan does not meet the objectives defined in Annex VII of Directive 2001/18/EC and the supplementing guidance notes (Council Decision 2002/811/EC). As a consequence a suitable update, which takes into account all relevant aspects, is highly recommended.

Therefore Austria is still of 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 its position also with new, additional scientific arguments.

As a consequence, Austria has set into force a national import ban on this product up to 1<sup>st</sup> October 2010. This timeframe should also be used for a scientific discussion on this transgenic maize line.

## ARGUMENTATION

### 1. Documents considered

The following argumentation is based on the documents provided by the notifier according to Directive 2001/18/EC as well as on previous statements of Austria during the notification procedure of maize MON 863. Additionally, it takes into account the responses of the notifier to the statements of the member states as well as the opinion of the German competent authority, opinions of the EFSA GMO-Panel, EMEA, WHO and relevant publications.

### 2. Introduction

Maize MON 863 contains the following DNA in two cassettes:

(a) Cassette 1:

A modified cry3Bb1 gene derived from *Bacillus thuringiensis subsp. kumamotoensis*, which confers resistance to the corn rootworm *Diabrotica spp.*, under the regulation of the 4AS1 promoter derived from Cauliflower Mosaic Virus, the wtCAB translation enhancer from wheat (*Triticum aestivum*), the transcription enhancer ract1 intron from the actin 1 gene of rice (*Oryza sativa*) and terminator sequences tahsp 17 3' from wheat.

(b) Cassette 2:

The nptII gene from *E. coli*, which confers resistance to aminoglycosides comprising kanamycin and neomycin, under the regulation of the 35S Cauliflower Mosaic Virus promoter, and the NOS 3' terminator sequences from *Agrobacterium tumefaciens* as well as the non-functional, truncated ble gene from *E. coli*.

The European Commission received the notification (Reference C/DE/02/9) for the placing on the market of the genetically modified maize MON 863 and the maize hybrid MON 863 x MON 810 for import and processing under part C of Directive 2001/18/EC from Monsanto on 7 February 2003, together with a positive assessment report from the lead member state (MS) Germany.

Austria as well as many other MS submitted its scientific concerns and objection to the placing on the market of this product to the Commission. The GMO-Panel adopted its opinion on the dossier on 2nd April 2004 and considered that the information available for MON 863 addresses the outstanding questions raised by the Member States during the approval procedure and concluded that MON 863 will not have an adverse effect on human and animal health or the environment in the context of its proposed use (EFSA (2004a)).

The opinion of the GMO-Panel is based on *in silico* and *in vitro* studies in the dossiers of the notifier, including the outcome of an acute toxicity study in mice and a 90 day rat study. Additionally, EFSA affirmed the safety of NPTII in its function as a selectable marker in genetically modified crops. The nptII gene codes for an aminoglycoside phosphotransferase conferring resistance to antibiotics such as kanamycin, neomycin, paromomycin, butirosin, gentamicin B and geneticin.

Austria - as well as many other member states - was of the opinion that the risks of this product on animal health and the environment were still not assessed properly by the notifier. As a consequence, Austria has – in line with a lot of other member states – voted against the placing on the market of this product during the Regulatory Committee acc. to Directive 2001/18/EC on 29<sup>th</sup> November 2004 and the Environmental Council on 24<sup>th</sup> June 2005, where a representative - though not qualified - majority could be reached (142 votes against the placing on the market). Nevertheless, the EC decided on 8<sup>th</sup> August 2005 in favour of this product, which got approval for placing on the market.

In autumn 2005 Austria informed the EC, EFSA and the CAs according to Directive 2001/18/EC about a scientific evaluation of the Monsanto report on the Subchronic Toxicity Study with maize MON 863 (BURNS (2002)): The general impression was that the subchronic toxicity study report lacks clarity, contains diffuse statements and has deficiencies in the performance. With regard to the study design it was stated that it was not designed to reveal a difference of possible toxic effects of the test diet compared to a control diet. Therefore, only effects with high incidences or great deviations from the control would have been detectable, with the chosen design. Concerning the OECD guideline as well as the applied statistical methods deficiencies were also detected and considered as not state of the art. Due to these lacks it was concluded that a repetition of the study is highly recommended.

The results were poorly described implying also the necessity of supplemental studies.

In 2006 Hammond et al. published a paper based on the of the same Monsanto 90-day rat feeding study but presented only a subset of the results provided by the Monsanto report (BURNS (2002), EFSA (2007b)). The authors referred in their paper additionally to studies on maize MON 863 in cattle (GRANT et al. (2003) and broilers (TAYLOR et al. (2003)) which were cited to support the absence of unintended changes induced by MON 863 on animal health. This conclusion seems to be difficult and lacks justification, as these studies that are mentioned above do not represent strictly speaking "toxicity" studies. A scientific discussion is provided in section 3.

In 2007 Séralini et al. published a statistical re-analysis of the data from the Monsanto 90-day feeding study as well as Monod, who used the same Gompertz curve as Séralini et al., but taking into consideration the variability between rats (SÉRALINI et al. (2007), MONOD H. (2007)).

Following this discussion, the GMO-Panel published an opinion on the analysis of data from a 90-day rat feeding study with MON 863 maize containing reviews of the previous documents and reports as well as an own extensive re-analysis of the original raw data (EFSA (2007b)), reaffirming the toxicological safety of MON 863 . Again a scientific discussion is provided under section 3.

In the Environmental Council held on 28<sup>th</sup> June 2007, 12 other member states supported the Austrian demand on a re-examination of maize MON 863 based on a state of the art risk assessment.

With regard to the nptII gene present in maize MON 863 as selection marker, EFSA concluded in its opinion that "the EFSA GMO Panel formulated already an Opinion (EFSA, 2004b) 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 (reviewed by BENNETT et al. (2004)). 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, 2004a).

In 2005 neomycin and kanamycin were classified as "critically important antibacterials" by WHO (2005), meeting the criteria of class 1 and 2 as in this document described:

**Criterion 1:** Sole therapy or one of few alternatives to treat serious human disease

**Criterion 2:** Antibacterial used to treat diseases caused by organisms that may be transmitted via non-human sources or diseases caused by organisms that may acquire resistance genes from non-human sources

In the WHO-paper (2005) it is argued that "A list of critically important antimicrobials for humans would facilitate the process of implementing specific management strategies to prevent the emergence and dissemination of resistance to those agents".

In respect with the authorisation of GM potato line EH92-527-1 under Regulation (EC) No 1829/2003 and Directive 2001/18/EC, which is also containing the nptII gene, EMEA was consulted by the Commission contradicting the arguments 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 all other issues raised by EFSA rebutting their arguments.

Thereafter "the GMO Panel agreed with the EMEA that the preservation of the therapeutic potential of the aminoglycoside group of antibiotics is important", but nevertheless re-affirmed in its additional statement 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 2007a).

In 2007 also a study on the "Risk Assessment of Antibiotic Resistance Marker Genes in Genetically Modified Organisms" (WÖGERBAUER, 2007) has been published, indicating a lot of deficiencies in the so far carried out risk assessment of ARM-genes, which is not carried out on a case-by-case basis, taking into account local differences in resistance levels and antibiotic usage patterns.

On 7th May 2008 the College 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 Amflora potato and three hybrid maize (MON863xMON810,

MON863xNK603, MON863xMON810xNK603) – all these products contain the nptII gene - the Commission will ask EFSA again to analyse further scientific evidence on the effects of these GMOs on the environment and human health. This underlines the present uncertainty in the EC with regard to ARM-genes present in genetically modified plants and strengthens the Austrian arguments that the use of nptII as selectable marker in GMOs cannot be regarded as state of the art. A detailed scientific discussion on this topic is given under section 4 of this document.

In section 5 the possibility of accidental spillage and the appropriate risk management measures are discussed.

Due to all these deficiencies in the risk assessment of maize MON 863 described and affirmed with new, additional scientific arguments, Austria has set into force a national import ban on this product up to 1<sup>st</sup> October 2010. This timeframe should also be used for a scientific discussion on this transgenic maize line.

### 3. Flaws in the toxicological risk assessment/possible adverse effects to animal health

#### 3.1. General remark

To date several animal studies with dairy cows (GRANT et al. (2003)) and fattening bulls (POL et al. (2005)), pigs (HYUN et al. (2005)), broilers (TAYLOR et al. (2003)) and rats (HAMMOND et al. (2006)) have been published investigating the impact of Cry3Bb1 on animal health.

Two approaches were used: most experiments investigated MON 863 under the aspects of substantial and nutritional equivalence (GRANT et al. (2003), POL et al. (2005), HYUN et al. (2005), TAYLOR et al. (2003)) one study was focussing on toxicological effects in rats (HAMMOND et al. (2006)). Studies referred to in some publications were not published in peer reviewed journals and are therefore not considered in this scientific argumentation. It has also to be stated that a majority of the published studies has been performed with scientists from Monsanto Company.

Because of the different experimental goals and designs the following considerations will be separated:

1. Studies on nutritional equivalence of transgenic crops, that were mainly performed in farm animals
2. Study on the toxicity of transgenic MON 863 in rats.

#### 3.2. Studies on nutritional equivalence assessment in farm animals

Several studies have been performed in farm animals using classical nutritional approaches to assess the nutritional equivalence of transgenic maize compared to conventional or near isogenic maize. The animal species used, comprise the range of farm animals and the study designs differ considerably. HAMMOND et al. (2006) refer to studies on MON 863 in cattle (GRANT et al. (2003)) and broilers (TAYLOR et al. (2003)), which are cited to support the absence of unintended changes of MON 863 on animal health. Therefore these studies are also evaluated in combination with other relevant literature in this respect.

##### 3.2.1. Study design

###### 3.2.1.1. Cultivation

Cultivation methods are important for the assessment of nutritional composition and equivalence studies. Reports on cultivations of MON 863 were different in extent, ranging from detailed description about growth and harvest (GRANT et al. (2003)) to short summarizing comments on this issue (POL et al. (2005), HYUN et al. (2005), TAYLOR et al. (2003)). Many of the so-called reference diets were not cultivated under strictly comparable conditions (TAYLOR et al. (2003)). Clearly defined and comparable conditions for the growth of the transgenic and isogenic plants have been given in the study with dairy cows by GRANT et al. (2003).

### 3.2.1.2. Experimental design of studies on nutritional equivalence assessment

The evaluated study designs are in accordance with scientific requirements for scientifically valid feeding trials using farm animals. These involve group design (control and test group), random assignment and replicates. Reference diets, reflecting a certain range of variability of normal, isogenic crops, were included in the test design (Table 1). However, since neither the cultivation conditions nor the nutritional equivalence of those crops are fully analogous, the test results can be difficult to interpret. This is especially a problem, when the sample size is not adjusted to the test group. Thus, the test and control groups represent often a population that is smaller compared to the reference groups. Reference groups are often contributing 60-80% of the sample size.

Statistically significant differences between test and control groups are therefore often masked because group differences between iso- and transgenic diets fall into the broad range of reference groups. This can be interpreted as follows: the positive assumption is, that the transgenic crop is clearly within the range of conventional maize varieties. The negative assumption would be, that differences between isogenic and transgenic maize are masked. Because of the character of the nutritional assessment of transgenic crops the aspect should normally have priority for the evaluation. Emphasis should be laid on the outcome of tests with closely related control groups (WEINGAND et al. (2002)).

Animals used in the studies were ruminants (dairy cows (GRANT et al. (2003) and fattening cattle (POL et al. (2005)) and monogastric animals (pigs (HYUN et al. (2005)), broilers (TAYLOR et al. (2003)) and rats (HAMMOND et al. (2006))). Using different species allows a broader and more comparative evaluation of potential effects by transgenic feed ingredients, which can be considered as positive for the assessment of GMOs and we would consider it to be preferable compared to using only one species. Except for cattle studies, all trials were performed using individuals of both sexes in an identical investigation scheme. Growing animals like steer calves and broilers after hatchery have high feed intakes, allowing the assessment of the GMO in a sensitive period with high growth rates.

Table 1: Overview of study designs of peer reviewed publications on MON 863 in farm animals

| Publication               | Species    | Group size                 | Control group  | Test group<br>MON 863 | Reference group(s) |
|---------------------------|------------|----------------------------|--|-----------------------|--------------------|
| <b>Grant et al., 2003</b> |            |                            |  |                       |                    |
| Exp. 1                    | dairy cows | n=16<br>(block design 4x4) | 1<br>(non transgenic control)                        | 0<br>(NK603)          | 2                  |
| Exp. 2                    | dairy cows | n=16<br>(block design 4x4) | 1<br>(non transgenic control)                        | 1                     | 2                  |
| <b>Pol et al., 2005</b>   |            |                            |  |                       |                    |
| Exp. 1                    | steers     | n=8                        | 1<br>(near isogenic, non transgenic parental hybrid) | 1                     | 0                  |

|                            |          |                                       |  |                                   |   |
|----------------------------|----------|---------------------------------------|--|-----------------------------------|---|
| Exp. 2;3                   | steers   | n=50 ; 49                             | 1<br>(near isogenic,<br>non transgenic<br>parental hybrid) | 1                                 | 2 |
| <b>Hyun et al., 2005</b>   |          |                                       |  |                                   |   |
| Study 1                    | pigs     | n=18 per sex<br>(block<br>design 2x4) | 1<br>(non transgenic,<br>genetically<br>similar)           | 1                                 | 2 |
| Study 2                    | pigs     | n=20 per sex<br>(block<br>design 2x4) | 1<br>(non transgenic,<br>genetically<br>similar)           | 1                                 | 2 |
| <b>Taylor et al., 2003</b> |          |                                       |  |                                   |   |
| Exp. 1                     | broilers | n=50 per sex                          | 1<br>(non transgenic<br>control)                           | 1                                 | 6 |
| Exp. 2                     | broilers | n=50 per sex                          | 1<br>(non transgenic<br>control)                           | 1<br>(MON<br>810 x<br>MON<br>863) | 6 |

### 3.2.1.3. Diets of studies on nutritional equivalence assessment

Verification of identity and the presence of the transgenic DNA by molecular tools and transproteins was performed by ELISAs (GRANT et al. (2003)) but the majority of the studies did not investigate or quantify the presence of genetic modification in the corn nor in the diet that was fed to the animals (POL et al. (2005), HYUN et al. (2005), TAYLOR et al. (2003)).

Moreover it has to be considered that crystal proteins can be degraded during processing. Ensiling markedly decreased the presence of long functional Cry1Ab gene fragments and full size Cry1Ab protein (LUTZ B. et al. (2006)). Additionally, pelleting has been shown to affect Cry proteins as they are heat labile (EPA (2003)). Pelleting can be performed at different conditioning temperatures (60°C -90°C). Without clear definition of the conditioning process it is not possible to predict the potential impact on DNA and proteins. Thus investigations of the modification should be done in the corn and in the end product, allowing complete assessment of the complete experimental diet (Table 2).

Table 2: Overview of diets used in nutritional equivalence studies published in peer reviewed journals

| Publication                | Duration      | Feed  | Processing | Test on identity |      |
|----------------------------|---------------|---|------------|------------------|------|
|                            |               |   |            | Grain            | Diet |
| <b>Grant et al., 2003</b>  |               |   |            |                  |      |
| Exp. 1                     | 21 d          | diet<br>40% corn silage<br>and 23.1% corn<br>grain  | ensiling   | ELISA            | no   |
| Exp. 2                     | 21 d          | diet<br>26.7% corn grain  | no         | ELISA            | no   |
| <b>Pol et al., 2005</b>    |               |   |            |                  |      |
| Exp. 1                     | 60 d          | grazing corn crop<br>residues plus<br>protein suppl.  | no         | no               | no   |
| Exp.2 & 3                  | 112d & 102 d  | diet<br>77.5% & 68.0%<br>corn grain   | no         | no               | no   |
| <b>Hyun et al., 2005</b>   |               |   |            |                  |      |
| Study 1                    | approx. 120 d | diet incl.<br>(68.7% grower1,<br>74.8% grower2,<br>78.7% finisher1,<br>82.5% finisher2)<br>corn grain | no         | no               | no   |
| Study 2                    | approx. 120 d | diet incl.<br>65.0% grower,<br>72% finisher1,<br>76% finisher2<br>corn grain                          | no         | no               | no   |
| <b>Taylor et al., 2003</b> |               |   |            |                  |      |
| Exp. 1                     | 42 d          | diets incl 55%<br>(starter) and 60%<br>(grower-finisher)<br>corn grain                                | no         | no               | no   |
| Exp. 2                     | 42 d          | diets incl 55%<br>(starter) and 60%<br>(grower-finisher)<br>corn grain                                | no         | no               | no   |

Diets were tested for proximate nutrient composition (GRANT et al. (2003), POL et al. (2005), HYUN et al. (2005), TAYLOR et al. (2003)), mineral and trace elements and selected amino acids (HYUN et al. (2005), TAYLOR et al. (2003)), (Table 3). No study investigated vitamin contents of grain or the complete diet.

Hygienic evaluation was very limited and included only mycotoxin analysis (GRANT et al. (2003), TAYLOR et al. (2003)) while other microbial traits were not measured. Only one study verified levels of pesticides (TAYLOR et al. (2003)). All diets were composed to meet the nutritional requirements of the investigated species. Since the main focus of the studies was on the nutritional effect of

different diets it should be the standard and state of the art to perform an extensive analytical investigation to avoid any disturbances from substances other than the intended modifications or unexpected and unintended ingredients.

Table 3: Analyses on corn and complete diets in peer reviewed MON 863 nutritional equivalence studies

| Publication         | Crude nutrients | Minerals & trace elements | Amino acids | Fatty acids | Hygienic evaluation (incl. mycotoxins) | Pesticides |
|---------------------|-----------------|---------------------------|-------------|-------------|--|------------|
| Grant et al., 2003  | yes             | Partial                   | no          | yes         | grain only                             | no         |
| Pol et al., 2005    | yes             | Partial                   | no          | no          | No                                     | no         |
| Hyun et al., 2005   | yes             | Partial                   | yes         | no          | No                                     | no         |
| Taylor et al., 2003 | yes             | Partial                   | yes         | no          | grain only                             | grain only |

#### 3.2.1.4. Endpoints and results of nutritional equivalence studies

All studies recorded performance characteristics as feed intake, body weight in the beginning and at the end of the study, and average body weight gain and feed conversion. Additionally, in dairy cows milk yields and milk composition were recorded (GRANT et al. (2003)). Steers, pigs and broilers were tested on carcass characteristics like muscle areas, rib fat (POL et al. (2005), HYUN et al. (2005)), back fat thickness (HYUN et al. (2005)), fat pad, chill, thigh, drum and wing weights (TAYLOR et al. (2003)) and meat quality analyses (POL et al. (2005), HYUN et al. (2005), TAYLOR et al. (2003)) .

Differences between the test and isogenic control groups were reduced dry matter intake, lower milk production and a negative body weight per period in dairy cows fed maize NK603 but not MON 863 (GRANT et al. (2003)). A significantly greater average daily gain ( $p=0.04$ ) and higher final live weights ( $p=0.03$ ) were seen in steers grazing on MON 863 corn crop fields (POL et al. (2005)). No differences in performance and carcass characteristics were found in broilers and pigs.

Table 4: Endpoints of studies on MON 863 in peer reviewed nutritional equivalence studies

| Publication                | Species    | Growth Performance | Carcass characteristics | Meat analysis | Additional analyses                     |
|----------------------------|------------|--------------------|-------------------------|---------------|---|
| <b>Grant et al., 2003</b>  | dairy cows | yes                | no                      | no            | Milk characteristics                    |
| <b>Pol et al., 2005</b>    | steers     | yes                | yes                     | yes           | Liver score, incidence of liver abscess |
| <b>Hyun et al., 2005</b>   | pigs       | yes                | yes                     | yes           | -                                       |
| <b>Taylor et al., 2003</b> | broilers   | yes                | yes                     | yes           | -                                       |

### 3.2.2. Discussion of nutritional equivalence studies

Studies on the nutritional equivalence of MON 863 compared to its isogenic control did not reveal clear and uniform differences between transgenic and conventional maize. Only one study in steers displayed significant differences in average daily weight gain and final body weight. Both traits were significantly higher in the MON 863 group (POL et al. (2005)). However, assessing the study, the *Bacillus thuringiensis* corn MON 863 used in this trial produced similar performance for both grazing and feedlot cattle compared with conventional corn.

Regarding the trend in all published papers, that the performance of animals fed with transgenic maize was similar to the performance of conventionally fed animals, allows the conclusion that the feeding value is probably not affected by the genetic modification. However, results are strongly associated with the methods used and the traits investigated and therefore the results have to be questioned.

It is well-known, that feeding trials in farm animals have a high variability. Therefore it is difficult to assess differences between closely related feed ingredients.

The aforementioned traits seem less appropriate for the assessment of closely related feedstuffs when the expected differences between test and control treatments are small. It is very likely, that results of performance trials do not indicate significant differences in such cases. The published experiments and feeding studies to test the nutritional value of transgenic feedstuffs were primarily based on recording feed intakes, growth rates, feed conversion and some also on the assessment of nutrient digestibility.

Due to the specific situation in farm animals with broad genetic variability and less standardized housing conditions it can be difficult to perform trials resulting in significant results due to the high intragroup variability.

In some of the published studies an incomplete characterization of feed composition is evident. This includes the aspects of identity of maize, hygienic

quality and the levels of pesticide residues, thus neglecting crucial procedures in feeding studies, which can not be regarded as state of the art.

### **3.2.3. Statistics of nutritional equivalence studies**

The use of hypothesis testing and reporting p-values in isolation has been widely criticised. P-values alone do not provide information about the size and direction of effect or the range of values. Thus, a significant result ( $p < 0.05$ ) could include clinically irrelevant differences, a non significant result ( $p > 0.05$ ) might hide an important difference that could exist but was not detected because of the study design and inappropriate endpoints investigated. So the use of confidence intervals might be better to provide information about significance and the size and direction of effect of the treatment compared with the control.

### **3.3. Studies on toxicity assessment in laboratory animals (Hammond et al. 2006; Burns (Monsanto report) 2002)**

One study in rats was carried out by Hammond et al. (2006). This is based on the original Monsanto study (BURNS J. M. (2002)) with the study title "13-Week Dietary Subchronic Comparison Study with MON 863 in Rats Preceded by a 1-Week Baseline Food Consumption determination with PMI Certified Rodent Diet #5002" but provided only a subset of the results provided in the Monsanto report (EFSA (2007b)).

A reanalysis of the existing data from this study was done by a French group of Séralini et al. (2007) using a different statistical approach model. The study involved a re-analysis of data from the Monsanto 90-day rat feeding study. These authors claimed to find statistically significant differences indicating liver and kidney toxicity in rats fed maize MON 863 and it was claimed by this group that MON 863 is not a safe product.

#### **3.3.1. Hammond et al. (2006)**

##### 3.3.1.1. Cultivation of MON 863 for toxicity assessment

The test, control and 3 out of 6 reference corn samples were planted in the same site. Three more reference maize samples came from a different region.

##### 3.3.1.2. Experimental design

The authors describe that the study design was based on the OECD Guideline No.408 (1981) for the testing of chemicals, section 4, health effects (also on p.14 of the Monsanto report) for a Repeated Dose 90-day Oral Toxicity Study in Rodents and in general compliance with OECD Good Laboratory Practice guidelines. This has to be criticised due to the fact that an outdated version of the respective OECD Guideline was used, omitting parameters that were already state of the art at the start of the Monsanto study. The study was started in March 2001 and at this time the revised OECD Guideline No. 408, adopted on 21<sup>st</sup> September 1998 was already published and available for two years. The updated OECD Guideline provides closer investigations of especially neurotoxic and immunotoxic effects.

Ten groups were involved in the study, among them 2 test groups fed on a diet formulated with either 11% or 33% MON 863 and 2 control groups, representing near isogenic control corn on the same dietary level (Table 5 and 6). In addition to this classical test and control design 6 reference diets on a 33% level were part of the study.

Ten groups of 20 Crl:CD (SD) IGS BR rats per sex and group were obtained from an established laboratory. Prior to the study the rats were fed the referring diets to assess the baseline food consumption.

Table 5: Overview of the study design based on the peer reviewed publication from Hammond et al. (2006) using inbred rats

| Publication          | animals | Group size   | Control group                | Test group MON 863 | Reference group(s) |
|----------------------|---------|--------------|------------------------------|--------------------|--------------------|
| Hammond et al., 2006 | Rats    | n=20 per sex | 2<br>(near isogenic control) | 2                  | 6                  |

### 3.3.1.3. Diets

The identity of the test grain was confirmed by MON 863 event-specific PCR analysis in the corn. Control grain was confirmed to be free of MON 863 events by the same method. The reference varieties were tested with different methods that were not clearly defined. PCR, ELISA or lateral flow stick methods can be used for that purpose (Table 6). These methods have different sensitivity, being lower for the lateral flow method and ELISA.

Unfortunately the authors did not mention if the rodent diets were pelleted or otherwise thermally treated or not. Usually rodent diets are pelleted. As pelleting induces temperatures up to 90°C and high pressure, a denaturation of protein occurs. Toxicity testing is only valid if it is guaranteed that the protein in the test material, the Cry3Bb1, is active and present in the diets. High emphasis should be laid on the diet preparation and a ground non heat treated diet seems therefore to be clearly preferable over pelleted or otherwise processed diets.

Table 6: Overview on diets and analytical characterization of diets used in the rat study of Hammond et al. (2006)

| Publication          | Duration | Feed                             | Processing | Test on identity  |              |
|----------------------|----------|----------------------------------|------------|---|--------------|
|                      |          |                                  |            | Grain   | Diet         |
| Hammond et al., 2006 | 90d      | diet<br>11% or 33% corn<br>grain | not clear  | PCR for test & control<br>Reference grain<br>either ELISA,<br>PCR or lateral<br>flow test | not<br>clear |

The diets for the rat study were prepared by PurinaLab Diets, a certificated manufacturer for laboratory diets. Diets were produced meeting specifications of Certified 5002 Rodent Diet, analytically confirmed by Covance Laboratories.

Only the grain and not the whole diets were analyzed for pesticide residues and mycotoxins (Table 7). Microbial counts considered as important hygienic traits were neither investigated in the grain nor in the diets.

Table 7: Analyses of the diets in the rat study of Hammond et al. (2006)

| Publication          | Crude nutrient s | Mineral & trace elements | Amino acids | Fatty acids | Hygienic evaluation (incl mycotoxins) | Pesticides |
|----------------------|------------------|--------------------------|-------------|-------------|---------------------------------------|------------|
| Hammond et al., 2006 | yes              | yes                      | yes         | yes         | grain only                            | grain only |

#### 3.3.1.4. Endpoints and results for toxicity assessment

The investigation scheme follows an outdated version of the OECD Guideline No.408 (see point 3.3.1.2.), which involves performance traits, clinical observations, haematology, serum chemistry, urine chemistry, necropsy and histological tests, which has already been criticised by Austria in 2005.

Adrenals, brain, heart, kidneys, liver, spleen, testes with epididymis, and ovaries were investigated for weight. Thymus and uterus weight evaluations were completely missing.

Additionally tissues were collected from animals fed 33% MON 863 and 33% control grain, including aorta, adrenals, bladder, brain, cervix, epididymides, esophagus, eyes, femur with joint and marrow, heart, intestine (ileum, jejunum, duodenum, colon, caecum), kidneys, lacrimal gland, lesions or abnormal masses, liver, lungs (with mainstream bronchi), lymph node (mesenteric), ovaries, pancreas, peripheral nerve (sciatic), pituitary, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle (thigh), skin (and mammary tissue in females), spinal cord (three levels), spleen, sternum with marrow, stomach, testes, thymus, thyroid/parathyroid, trachea, uterus and vagina.

Following collection, tissues were placed directly into 10% neutral buffered formalin for fixation. Adrenals, brain, colon, duodenum, heart, ileum, jejunum, kidneys, liver, lymph node (mesenteric), ovaries, pancreas, rectum, spleen, stomach, testes, and thyroid/ parathyroid were examined histologically from all animals. Only around half of the organs required by the Guideline and tissues were analysed histopathologically.

There was no report on the microscopic examination of the bone marrow, though it was collected. The investigation of the bone marrow could have been supplementary to the haematology showing differences between the groups. Also ophthalmoscopic investigations were not performed.

Significant but transient differences were found in female rats of the 33% MON 863 group that had a higher weight gain in the 3<sup>rd</sup> week and a lower in the 4<sup>th</sup> week as compared to the control. Males fed the MON 863 diet had a significantly lower feed consumption at week 3 and 10 compared to the control group.

Haematology and clinical biochemistry in blood samples were characterized by some differences between groups, however, with high variability. The haematological results indicated a significant increase of white blood cell counts

in males of the 33% MON 863 group due to an increase of lymphocytes and basophiles.

Significantly higher glucose levels were seen in females fed the 11% and 33% MON 863 diet and significantly lower chloride and higher sodium levels in males assigned to the high and low dose MON 863 group, respectively. Significant changes in triglyceride and globulin levels (leading to a significantly lower albumin/globulin ratio) were seen in females that had 11% genetically modified corn in their diet. In urine analyses the urine specific gravity was lower in males fed 11% MON 863 than the control males.

No differences were seen at necropsy and histological examination, except for kidneys that showed a significantly higher tubular mineralization in females of the high dose MON 863 group as compared to the control group.

### **3.3.2. Supplementary considerations to Hammond et al. (2006) based on the original Monsanto report**

The original Monsanto report was carried out by BURNS (2002). The paper contains some additional information and considerations compared to the peer reviewed publication of HAMMOND et al. (2006).

General and structural appearance of the report is impaired as many supplementary papers were submitted and the data presentation is therefore not very transparent for the reader. This is supported by an unclear collation of the various contributions. Also the many deviations of the protocol give the study a difficult appearance.

With regard to the already mentioned criticism of using an outdated OECD Guideline (see point 3.3.2.1. and 3.3.1.4.), following **deviations** from the respective OECD Guideline have to be mentioned:

- a) Dose groups:  
OECD requires 3 dosed groups whereas in the study only 2 dosed groups (11 and 33 % corn in the diet) were used. This deviation is acceptable, as no major toxicological effects were expected. In the case that toxic effects would have been observed, the study had to be repeated with at least 3 doses.
- b) Highest dose:  
The highest feasible dose should be used if no toxic effects are expected. It is not clear to the reader and no rationale is found in the report that 33 % maize corn in the diet is the highest possible and reasonable dose. No justification is given for this dose except that the nutritional composition of the various diets used was matched to a specific commercial rodent diet. And even this explanation is not found (at least not in the main part) in the report itself but only in one of the supplementing papers (LEMEN et al. (2002)).
- c) Room temperature:  
OECD requires an ambient temperature for the rats of  $22 \pm 3$  °C, whereas 18 to 26 °C were set (not: registered) for the study. Apart from this deviation it is reported that "variations to these conditions are documented

in the raw data", indicating that further deviations, beyond 18 to 26 °C, have occurred.

d) Housing of animals:

The cage size is not reported. The hygienic status of the animal rooms, e.g. SPF-conditions or open conditions, is not described.

e) Form of the diets:

Little information is available on diet pre-treatment and feed technology used. As in the HAMMOND-paper the question remains open, whether the diets were pelleted or otherwise heat treated. Hygienic processes including vigorous heat treatments are common in diets for laboratory animals and could have affected the results. The type of container for the diet within the cage is also not described.

f) Justification of the control item:

There is no rationale for the selection of the maize hybrid LH82xA634 as the closest related non-transgenic line to MON 863. It is only stated that the "background genetics is representative of event MON 863". From the EFSA evaluation (EFSA, 2004) it is learned that MON863 was generated from the cell line AT824. Therefore AT824 would be the closest related non-transgenic maize line and the appropriate control to MON 863.

g) Justification of the reference items:

There is no rationale for the selection of the specific 6 commercially available maize lines included in the reference diets.

h) Stability of the diet:

The stability of the diets was not investigated, only information (stability for 6 months) of the supplier for a commercial rodent diet was used also for the other diets.

i) Animal observation:

The extent of the animal observation remains unclear. Investigations for assessing sensory reactivity to stimuli, grip strength and motor activity were not performed.

j) Ophthalmoscopy:

Ophthalmological investigations are required by OECD, but were not performed in the study.

k) Organ weight:

OECD requires the determination of the organ weight also of uterus and thymus, which is missing in the study.

l) Histopathology:

OECD requires the histopathological analysis of around 40 organs and tissues and not only of 17 as in the study described. Histopathological analyses are often the most sensitive investigations within a repeated dose toxicity study. A reduction of the extent of these analyses is a major deviation.

### 3.3.2.1. Statistics

The study was designed to compare maize MON 863 with various non-transgenic maize varieties in diets. These various non-transgenic diets give a scattering of responses of the test organism and the hypothesis of the study was to demonstrate that the responses due to MON 863 are within the distribution of the responses of the other maize varieties.

Accordingly, the statistical analysis relies on three criteria to label a result as statistically significant different, compared to the other groups. Each of the three following criteria has to be fulfilled:

- 1) The overall analysis of variance of the 10 groups indicates significant differences.
- 2) The difference between the test group and the mean of all reference diets or the control diet is significant.
- 3) The mean of the test diet is outside the range of the means of the reference or control diets.

(For comparison: A study to reveal toxic effects of an e.g. chemical would rely statistically mainly on "The test group is significantly different from the corresponding control group - or not".)

The inclusion of the 6 reference maize lines produces a relatively large distribution of responses of the test organism and due to the application of the sum of the three criteria, only a few significances remained. Therefore it is not surprising that significant differences between the test group and the corresponding control group were masked in the large distribution of responses and were not discussed in the report:

- a) First examples: The male body weight gain in the first weeks was significantly lower in the low dose test group than in the corresponding control group (see pages 1002-1003 of the report), but these differences were not discussed. In the main part of the report, on page 25, it reads "No test-article-related differences in body weight or changes in body weight were observed".
- b) Second example: The reticulocyte count of females in week 14 was significantly lower in the high dose test group than in the corresponding control group (and even lower than in all the 6 reference groups), see page 1042 of the report. Nevertheless this difference is not discussed, because the analysis of variance of the 10 groups just missed the significance level. The main part of the report says (page 25-26): "There were no alterations in the haematology and coagulation data ... that would indicate an effect from the feeding of any of the test diets".

### 3.3.3. Conclusions from the Toxicity study

HAMMOND et al. (2006) concluded, that the significant differences were not product-related as they were transient, not dose related, of small magnitude and not present in both sexes.

It is clear that dose-related effects between treatment groups and assessment of identical findings in both sexes can strongly support a clear interpretation. This was not obvious in this study. Another criterion for a toxicologically important effect is the reproducibility of the findings (WILSON et al. (2001)). Ideally, this does not only affect males and females or high and low dose groups but the outcomes have to be repeatable in independent experiments. This is especially relevant for the interpretation of studies with an outcome that is not clear regarding its biological significance. The general impression that the experimental design was not really straightforward is underlined by the original Monsanto report.

HAMMOND et al. (2006) refer to studies on MON 863 in cattle (GRANT et al. (2003)) and broilers (TAYLOR et al. (2003)), which are cited to support the absence of unintended changes of MON 863 on animal health. This conclusion can again not be regarded as appropriate due to the many deficiencies mentioned (see point 3.2.) and additionally lacks justification, as these studies do not represent strictly speaking "toxicity" studies.

HAMMOND et al. (2006) refer also to an acute mouse gavage study which is supposed to be an adequate test model for acute toxicity testing. They consider these acute toxicity studies to be appropriate to identify the potential risk of Cry proteins. This statement can be considered as weak due to the low level of Cry proteins in plants. A repeated dose 28-day Oral Toxicity Study in Rodents test design as proposed in the OECD Guideline No. 407 (1995) is regarded as more suitable.

An important factor is also the sensitivity of the animal model. HAMMOND et al. (2006) used an outbred rat model.

Additionally the study compared a high number of different lines of maize, among them MON 863. Further more there is no sufficient justification for the selection of the control maize line LH82xA634.

The data vary considerably in and between the groups. This leads to the assumption that only effects with great deviations from the control would have been detectable with the chosen trial setup. The choice of inbred strains would have resulted in lower variability remains questionable. Inbred rat strains have the advantage to reduce individual differences but might carry a risk of lower sensitivity of the chosen genotype. When using an inbred strain, several inbred subgroups might give a broader view on diet-genotype interaction. This aspect has to be considered when planning and performing feeding trials.

Due to the above mentioned deficiencies, a complete repetition of the Monsanto report by independent scientists is highly recommended.

Finally, toxicity studies are performed to estimate whether a test substance causes biologically important effects on animal and further on human health. This important issue can not only be investigated in toxicity studies in the target

species. A broader view on that issue and the summation of findings from different aspects - like environmental risk – resulting in decision-making based on a holistic approach, should be made.

#### **3.3.4. Reanalysis of data published by Hammond et al. (2006)**

The difficulty in risk evaluation was shown by a working group of Séralini who re-analysed the data of the 90 day rat study. The authors pointed out a large number of significant results and referred for this to HAMMOND et al. (2006), who provided only a subset of the results provided in the original Monsanto report (BURNS (2002), EFSA (2007b)): Séralini et al. revealed some differences in body weight and haematology as well as clinical chemistry data using a specific statistical approach applied to the same primary data.

The Commission du Génie Biomoléculaire (CGB) in France commissioned a statistical analysis of the weight data from the 90 day study. For this purpose MONOD (2007) provided an extensive statistical analysis of the MON 863 data: Subsequently MONOD (2007) used the same Gompertz curve as SÉRALINI et al. (2007), but taking into consideration the variability between rats (EFSA, 2007b).

After a reassessment of the statistical methodology by EFSA (2007b), pointing out the advantages and disadvantages of the two different statistical models and also containing an own extensive re-analysis of the original raw data, it was concluded by the GMO-Panel that maize MON 863 is further considered as safe product. Additionally a review article of an Expert Panel of toxicologists and statisticians from North America and Europe (DOULL (2007)) supported the EFSA opinion and concluded that the Séralini-study did not reveal any new scientific data on any adverse effects of Cry3Bb1.

However it has to be stated in this respect that no new data have been investigated but only statistical re-evaluations of the (original) data have been carried out. These data were not generated applying state of the art methodology – as previously described – and as a consequence all statements ensuring the safety of the product, which are based on these data cannot be regarded as sound scientific proof for the human and animal safety of maize MON 863.

#### **3.4. General Conclusion**

The risk assessment of genetically modified food and feed is a case-by-case decision. The study designs and methods require careful interpretation of results.

With regard to the **studies on nutritional equivalence assessment in farm animals**, which are quoted in HAMMOND et al. (2006) as scientific proof for the safety of maize MON 863, a lot of deficiencies have been detected:

- a) Concerning the experimental design it has to be criticised that reference groups are often contributing 60-80% of the sample size. Statistically significant differences between test and control groups are therefore often masked because group differences between iso- and transgenic diets fall into the broad range of reference groups. Emphasis should be laid on the outcome of tests with closely related control groups.

- b) With regard to diets used, it has to be critically noted that the majority of the studies did neither investigate nor quantify the presence of GMO-contamination in the corn, nor in the diet that was fed to the animals.
- c) Furthermore a clear definition of the conditioning process is missing, which makes it impossible to evaluate the potential impact on DNA and proteins. Thus investigations of the modification should be done in the corn and in the end product, allowing complete assessment of the complete experimental diet.
- d) No study investigated vitamin contents of grain or the complete diet.
- e) Hygienic evaluation was very limited and included only mycotoxin analysis (GRANT et al. (2003), TAYLOR et al. (2003)) while other microbial traits were not measured. Only one study verified levels of pesticides.

However, results are strongly associated with the methods used and the traits investigated. Therefore the results of these studies have to be questioned and cannot be regarded as a scientific proof for the safety of maize MON 863. Additionally these studies cannot be used for statements or citations on chronic toxicity.

With regard to the **studies on toxicity assessment in laboratory animals** it has to be stated that also there many deficiencies are obvious:

- a) An outdated version of the OECD Guideline No 408 was used, omitting parameters that were already state of the art two years in advance of the beginning of the Monsanto study. Therefore a lot of deviations in the study design are obvious, which cannot be regarded as appropriate.
- b) Concerning the description of the diets used (HAMMOND et al. (2006)), it has to be remarked that the reference varieties were tested with different methods, which were not clearly defined: The authors did not mention if the rodent diets were pelleted or otherwise thermally treated or not.
- c) Additionally microbial counts, which are considered as important hygienic traits, were neither investigated in the grain nor in the diets.
- d) Further more there was no report on the microscopic examination of the bone marrow, though it was collected. The investigation of the bone marrow could have been supplementary to the haematology showing differences between the groups.
- e) Thymus and uterus weight evaluations were completely missing. This can not be regarded as appropriate.
- f) An important factor is also the sensitivity of the animal model: HAMMOND et al. (2006) described the use of an outbred rat model. The study compared a high number of different lines of maize, among them MON 863. The data vary considerably in and between the groups. That would allow the assumption that only effects with great deviations from the control would have been detectable with the chosen trial setup.

With regard to supplementary considerations to HAMMOND et al. (2006) based on the **original Monsanto report** it has to be mentioned, that the data are not presented in a user friendly way: The report consists of an unclear collation of the various contributions. Also many deviations of the protocol give the study a difficult appearance.

- a) Again the use of an outdated version of the OECD Guideline No. 408 has to be criticised.
- b) Furthermore animal husbandry is insufficiently described.
- c) Little information is available on diet pre-treatment and feed technology used. As in the HAMMOND-paper, the question remains open whether the diets were pelleted or otherwise heat treated.
- d) GRANT et al. and TAYLOR et al. are cited to support the absence of unintended changes of MON 863 on animal health. This conclusion can again not be regarded as appropriate due to the many deficiencies mentioned above and additionally lacks justification, as these studies do not represent strictly speaking "toxicity" studies.
- e) The statistical methods used in the Monsanto report can not be regarded as suitable for detecting differences in the response of the rats to the diet compared to the control diet.

Concerning the **re-analysis of data** it has to be mentioned that the EFSA analysis did not find any consistent pattern over dose and gender rendering changes of biological importance. But these findings are based on data, which were generated in a not state of the art way. Also all deficiencies identified and concluded in this scientific statement have to be taken into consideration. Therefore these data can not be regarded as a suitable scientific proof for the human and animal safety of maize MON 863.

### **3.5. Recommendations**

- a) Due to the above mentioned deficiencies, a complete repetition of the Monsanto report by independent scientists is highly recommended.
- b) Investigations with maize MON 863 should include elaborate designs including sensitive periods as reproduction or ageing.
- c) Research by independent scientists and research groups should be supported in the future.

## 4. nptII-gene

### 4.1. General remark

In the following section the arguments of the notifier and their affirmation of 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.

### 4.2. Therapeutic relevance of kanamycin, neomycin and paromomycin in human and veterinary medicine

In the dossier a draft version of a study with regard to “The safety of nptII for human health and the environment” (GAY, submitted 2003) is attached, concluding that “the three antibiotics to which the nptII gene confers resistance, namely kanamycin, neomycin and paromomycin are disappearing from the market, particularly in the EU. Their toxicity has always been a major limitation to their use in human medicine. In the EU, while the use of the older antimicrobials (mainly neomycin) is still permitted in veterinary medicine, none of the three are presently used either orally or parenterally in human medicine.” This statement does neither reflect the de facto importance of these antibiotics in human and veterinary treatment nor the actual size of application within the EU. This is underlined by the following scientific arguments:

#### 4.2.1. Human medicines considerations

The therapeutic relevance of kanamycin and neomycin in human medicine has also been addressed by the GMO panel of EFSA. But the EMEA/CHMP considered in their statement (EMEA, 2007) a more long-term view recognising the potential development in the aminoglycoside class indicating that the role of these medicinal products might become increasingly relevant:

- Aminoglycosides belong to a class of antibiotics that has become increasingly important in the prevention and treatment of serious invasive bacterial infections in humans. This is because gram-negative bacteria (and tuberculosis bacteria) are becoming resistant to other classes of antibiotics.
- Aminoglycosides such as kanamycin are currently recommended for treatment in multidrug resistant tuberculosis (MDR-TB). Drug resistance in TB is part of the explanation for the resurgence of TB. WHO estimates that eight million people get TB every year. In the absence of an effective therapy, infectious MDR-TB patients will continue to spread the disease, producing new infections with MDR-TB strains. Until the introduction of a new drug with demonstrated activity against MDR strains, this aspect of the TB epidemic could begin to explode at an exponential level (from the Global Alliance for TB Drug development (<http://www.tballiance.org>)). In Estonia, Kanamycin was very recently introduced in the TB program (EMEA, 2007).

The EMEA also included kanamycin for potential use in MDR-TB in the case of bioterrorism

(<http://emea.europa.eu/pdfs/human/bioterror/11.Otherbacterial.pdf>)

In its opinion (EFSA, 2007a) the GMO Panel agreed with the EMEA that the preservation of the therapeutic potential of the aminoglycoside group of antibiotics is important. But EFSA maintained its opinion “that the therapeutic effect of these antibiotics will not be compromised by the presence of the nptII gene in GM plants, given the extremely low probability of gene transfer from plants to bacteria and its subsequent expression”. Furthermore the GMO Panel reiterated its earlier conclusions (EFSA, 2004b)

But the proposition of the notifier that most of the antibiotics which are inactivated by ARM gene encoded proteins are outdated, of no clinical use and/or replaced by more effective and less toxic antimicrobials as well as the statement and literature cited in the EFSA opinions that an introduction of additional resistance functions by ARM genes from transgenic plants would not lead to significant disturbances of everyday antimicrobial chemotherapy (BENNETT et al. (2004), EFSA (2004b), GOLDSTEIN (2005) in WÖGERBAUER, 2007 ) can not be regarded as appropriate. These generalizing assumptions are challenged by the observation of substantial differences in antibiotic usage patterns and consumption throughout Europe (MONNET and LOPEZ-LOZANO (2005), NIELSEN et al. (1998)) in WÖGERBAUER, 2007).

Table 8 displays all relevant indications of antibiotics inactivated by the nptII-ARM gene (WÖGERBAUER, 2007):

Antibiotic resistance marker genes – relevance of the corresponding antibiotics

| Resistance gene     | Resistance           | Inactivated antibiotics   | Human therapeutic applications of the corresponding antimicrobials   |  |                                    | Veterinary medicine  |           |
|---------------------|----------------------|---|--|--|------------------------------------|--|-----------|
|                     |                      |   | Range of use   | Austria (42) <sup>1)</sup>   | Relevance <sup>2)</sup>            | Range of use   | Relevance |
| nptII<br>aph(3)-IIa | kanamycin resistance | kanamycin   | second-line antibiotic for multidrug resistant mycobacteria (antituberculosis drug) (11)<br>topical application with skin, eye and ear infections  | no indication  | minor (critically important) (324) | infections in cattle, sheep, goats, pigs, dogs and cats (69)           | minor     |
|                     |                      | neomycin  | bad oral absorption, in rare cases intravenous/intramuscular application; preoperative gut sterilisation, selective bowel decontamination with high risk patients; hepatic encephalopathy  | topical application: infections of skin and mucosa<br>oral: no indications   | minor (critically important) (324) | enteritis of calves, pigs, poultry<br>eczema, dermatitis of cats, dogs | minor     |
|                     |                      | gentamicin = G418   | exclusive application as selective agent in <i>in vitro</i> experiments<br>no clinical usage   | no application   | none                               | no application   | none      |
|                     |                      | (paromomycin) <sup>4)</sup>                                     | selective bowel decontamination  | hepatic encephalopathy, preetozoma, coma; preoperative reduction of the bowel flora, intestinal amoebiasis   | minor                              | trichomoniasis in pigeons (69)   | minor     |
|                     |                      | (butirosin) <sup>4)</sup>                                       | n. a.  | n. a.  | n. a.                              | n. a.  | n. a.     |
|                     |                      | (ribostamycin) <sup>4)</sup>                                    | n. a.  | n. a.  | n. a.                              | n. a.  | n. a.     |
|                     |                      | (gentamicin B) <sup>4)</sup>                                    | severe nosocomial infections by Gram negative bacteria (sepsis, endocarditis, pneumonia), see gentamicin (203)   | always in adjunction: infections due to Gram negative rods<br>monotherapy only: urinary tract infections   | high (critically important) (324)  | n. a.  | n. a.     |
|                     |                      | (amikacin: <i>in vitro</i> inactivation by nptII) <sup>4)</sup> | important second-line antibiotic after treatment failure with other aminoglycosides (11),<br>important second-line antibiotic for hospital acquired infections due to Gram negative bacteria, which are resistant to gentamicin and tobramycin | used for bacterial strains which are resistant to other aminoglycosides (esp. gentamicin)<br>first line drug for patients who suffer from severe immunodeficiency/sepsis mycobacteria, if resistant to other antibiotics | high (critically important) (324)  | n. a.  | n. a.     |

<sup>4)</sup> The variant of nptII usually used as marker gene in plants does not confer resistance to those antibiotic compounds placed in brackets (EFSA, 2004b).

With regard to the conclusion of the notifier and EFSA that neomycin and kanamycin are of minor therapeutic importance, it has also to be stated that the terms “infrequent use” and “limited indications” cannot be equated with “minor therapeutic importance”. The use may remain infrequent, but the importance of use of neomycin and kanamycin for decolonisation/decontamination may well increase as a consequence of increasing problems with multiresistant or panresistant gram-negative bacteria and of multiresistant staphylococci (EMEA, 2007).

In the year 2005 WHO classified neomycin and kanamycin as “critically important antibacterials”, meeting the criteria of class 1 and 2 as in this document described:

**Criterion 1:** Sole therapy or one of few alternatives to treat serious human disease

Therefore it is self-evident that these antimicrobials have an important place in human medicine. It is of prime importance that the utility of such antibacterial agents should be preserved, as loss of efficacy in these drugs due to emergence of resistance would have an important impact on human health.

**Criterion 2:** Antibacterial used to treat diseases caused by organisms that may be transmitted via non-human sources or diseases caused by organisms that may acquire resistance genes from non-human sources

Antibacterial agents used to treat diseases caused by bacteria that may be transmitted to man from non-human sources are considered of higher importance. In addition, commensal organisms from non-human sources may transmit resistance determinants to human pathogens and the commensals may themselves be pathogenic in the immunosuppressed. The link between non-human sources and the potential to cause human disease appears greatest for the above bacteria.

The history of the development of antimicrobial resistance shows that resistance may appear after a long periods of usage (e.g. vancomycin resistance in *Enterococcus faecium* was first detected after the drug had been in use for over 40 years). If resistance has not developed to date it does not assure that it will not develop in the future (WHO, 2005).

In the WHO-paper (2005) it is therefore argued that “A list of critically important antimicrobials for humans would facilitate the process of implementing specific management strategies to prevent the emergence and dissemination of resistance to those agents”.

#### 4.2.1.1. Therapeutic relevance of the corresponding inactivated antimicrobials

In several countries kanamycin is kept as second line antibiotic for the treatment of multi-drug resistant tuberculosis such as in Estonia or the USA (BADDOUR and GORBACH (2003) in WÖGERBAUER, 2007).

In rare cases neomycin and paromomycin are used orally for pre-operative bowel sterilization or selective gut decontamination in high-risk patients or for the treatment of hepatic encephalopathy (Chemotherapie, Ö. G. f. (2007), EFSA, (2004b) in WÖGERBAUER, 2007).

All other applications are limited to topical administrations with skin, eye and ear infections (e.g. otitis externa, conjunctivitis) (BADDOUR and GORBACH (2003), Chemotherapie, Ö. G. f. (2007), EFSA (2004b), GOLDSTEIN et al. (2005) in WÖGERBAUER, 2007).

Though these antibiotics are actually of minor therapeutic relevance in human therapy in Austria, in many EU-member states - such as France, Italy, Germany, Lithuania and Estonia – these antibiotics are licensed and used (EMEA, 2007; WÖGERBAUER, 2007)

#### 4.2.2. Veterinary usage consideration

As mentioned earlier, the use of neomycin and kanamycin is currently limited, but this does not equate to “minor therapeutic importance”. Importance is not measured by the quantity used, but rather relates to the need for the antibiotic and what alternatives exist, if any. As resistance continues to increase to the alternative drugs, the importance of neomycin and kanamycin and future derivatives of these drugs can be expected to increase, e.g. therapy of neonatal diarrhoea in piglets and treatment of multi-resistant enteric gram negative bacteria (EMEA, 2007).

Kanamycin and neomycin are also included in the OIE list of VCIA. For aminoglycosides the following is indicated:

“The diseases controlled by aminoglycosides, either alone or in combination, are particularly debilitating to young animals and failure to adequately treat outbreaks would result in much suffering among affected animals. Similarly, the enteric infections affecting pigs and calves are effectively and economically treated orally with aminoglycosides, either alone or in combination. The wide range of applications and the nature of the diseases treated make aminoglycosides critically important for veterinary medicine and animal production”. (EMEA, 2007)

The following aminoglycosides are part of veterinary medicines for food producing species in the EU: apramycin, dihydrostreptomycin, gentamicin, kanamycin, neomycin, paromomycin, spectinomycin and streptomycin. (EMEA, 2007)

##### 4.2.2.1. Therapeutic relevance of the corresponding inactivated antimicrobials

Neomycin is used for the treatment of enteritis in calves, pigs and poultry and sometimes for skin infections in cats and dogs (EFSA, 2004b)). In Austria four kanamycin containing preparations are licensed and several preparations with neomycin are available. Paromomycin is used for the treatment of infections in pigeons (see table 8) (WÖGERBAUER, 2007).

In other EU - member states neomycin and kanamycin are also used regularly:

- **France:** neomycin is used regularly for treatment of diarrhoea in pigs and is not a minor drug. The level of resistance varies between 5 and 20 % in *E. coli* samples in the French monitoring programme (see FARM report4). Sales of aminoglycosides in 2005 in France were 77.8 Tonnes (5.89% of total sales).
- **Denmark:** the use of aminoglycosides in piglets and calves in Denmark is almost exclusively restricted to neomycin.
- **Germany:** the following number of products are licensed:
  - Gentamicin: Veterinary medicine 25
  - Kanamycin: Veterinary medicine 1

- Neomycin: Veterinary medicine 30
- **The Netherlands:** neomycin is used for oral treatment of enteritis in pigs and calves, for Bovine Respiratory disease by injection in combination with benzylpenicillin, for local treatment of mastitis (always in combined with a beta-lactam) and in eye and ear ointments. Specifically the parenteral use of neomycin-penicillin is substantial.
- **Spain:** different Marketing Authorisations for veterinary medicinal products from different Laboratories exist.
- **Ireland:** kanamycin is used in the treatment of mastitis in cows. (EMEA, 2007)

#### 4.2.3. Overall conclusions from human and veterinary use

For a risk assessment of ARM genes it should be taken into account that transgenic DNA survives the passage through the mammalian gastrointestinal tract, although fragmented to some degree and may be transferred to gut bacteria or intestinal epithelial cells (FAO/WHO, 2001 in WÖGERBAUER, 2007). Attention should be paid to the specific characteristics of the encoded DNA sequences, the properties of the receptor organisms and the prevailing selective pressures in the environments they reside. Several FAO/WHO working groups recommend sparing ARM genes and other non-essential sequences which could stimulate transfer or recombination. Because the transfer of ARM genes to intestinal bacteria cannot be excluded a priori the relevance of the corresponding inactivated antibiotics for human or animal therapeutic applications have to be taken into account (FAO/WHO, 2001 in WÖGERBAUER, 2007).

Therefore it can be concluded – as stated already by EMEA (2007) - that neomycin and kanamycin are of importance for veterinary and human use and that their current and potential future use cannot be classified as of no or only minor therapeutic relevance.

#### 4.3. Kanamycin and neomycin resistance in natural environments

It is argued by the notifier and EFSA that neomycin/kanamycin resistance is already widespread in the bacterial community, being present in a number of pathogenic bacteria, in many animal and human bacterial commensals and in soil bacteria. It is concluded that the nptII gene therefore poses no risk to modern aminoglycoside based human therapy (GAY, submitted 2003).

This general statement cannot be regarded as appropriate due to the following reasons:

Resistance to aminoglycoside antibiotics can be mediated by at least three different mechanisms:

- 1) Single-step mutations in the ribosomal rRNA target or in genes coding for ribosomal proteins render cells insensitive to the antibiotic. This event may occur at a frequency of 1 mutation every  $10^8$  cell divisions (GILLESPIE (2002), RAMASWAMY and MUSSER (1998) in WÖGERBAUER, 2007).
- 2) Uptake of the antibiotic may be impeded by an efflux pump which readily removes the antimicrobial from the cytoplasm protecting the ribosomal target from lethal concentrations (VOGNE (2004) in WÖGERBAUER, 2007).

- 3) Uptake of resistance determinants coding for aminoglycoside modifying enzymes (belonging to at least 11 different gene families) by horizontal gene transfer (GAY and GILLESPIE (2005) in WÖGERBAUER, 2007).

In a later publication GAY and GILLESPIE (2005) concluded that overall resistance mediated by nptII appears to have little, if any, clinical significance.

But literature survey reveals that only few studies are available to provide accurate data on the environmental distribution of the nptII gene in Europe. Most of the available peer-reviewed reports suggest a very low prevalence of the nptII gene among bacteria of non-clinical origin (LEFF et al. (1993), SMALLA et al. (1993) in WÖGERBAUER, 2007).

LEFF et al. reported on the situation in the USA, SMALLA et al. obtained limited samples sets from the Netherlands and Germany (GEBHARD and SMALLA (1998), SMALLA et al. (1993) in WÖGERBAUER, 2007). No nptII genes were detected in soil samples, but nptII was present in sewage and manure samples. Only a marginal proportion of insensitive clinical strains carried nptII as resistance determinant (SHAW (1993) in WÖGERBAUER, 2007). The most prominent carriers are particularly Gram-negative bacteria like *Pseudomonas spp.*, *Aeromonas spp.* and *E. coli* (SMALLA et al. (1993) in WÖGERBAUER, 2007). NIELSEN et al. (2005) were not aware of any peer-reviewed study which has dealt systematically with the environmental presence of nptII genes in Scandinavia (in WÖGERBAUER, 2007).

The phenotypic level of kanamycin resistance (which provides no information about the genes responsible for it) is rather widespread, with approx.  $10^5$  resistant bacteria per gram of soil (HENSCHKE and SCHMIDT (1990), SMALLA et al. (1993) in WÖGERBAUER, 2007). In the USA, a low level of phenotypic resistance to kanamycin was reported in litter of poultry (KELLEY et al. (1998)) and fresh water samples (KELCH and LEE (1978) in WÖGERBAUER, 2007). A study analyzing the resistance profiles of *Campylobacter jejuni* isolates from wild birds did not detect any neomycin resistant isolate, suggesting a low prevalence of the nptII gene in an avian reservoir of these enteropathogenic bacteria (WALDENSTROM et al. (2005) in WÖGERBAUER, 2007).

In Norway resistance to neomycin was also marginal, reaching a top value of only 3% of nonsensitive *E. faecalis* isolates from faecal poultry sources in the observation period from 2000 to 2003. Most of the other tested isolates were completely sensitive to this antibiotic. Although data on aminoglycoside resistance of *E. coli* samples were scarcely, only 3.2% of pork samples showed resistance to kanamycin. Resistance to neomycin was between 0 and 2% of the isolates. Summarizing these Norwegian data, an extremely low incidence of kanamycin, respectively neomycin resistance in "environmental" bacterial samples can be reported (NIELSEN et al. (2005) in WÖGERBAUER, 2007).

Moreover, recent reports also indicate a low background level of aminoglycoside resistance functions in certain bacterial strains (GIBREEL et al. (2004), HAUSCHILD et al. (2007), SCHMITZ et al. (1999) in WÖGERBAUER, 2007). "Environmental" enterococci isolates from wild boars in Portugal revealed kanamycin (mediated by nptIII) and streptomycin resistance rates of a meager 9% and 6.7%, respectively (POETA et al. (2007) in WÖGERBAUER, 2007). In commercialized poultry samples an elevated incidence of resistance to

kanamycin (30%) was reported, reflecting different selection pressures active in different environments (NOVAIS et al. (2005) in WÖGERBAUER, 2007). Dairy isolates of enterococci were mostly susceptible to gentamicin and kanamycin, and clinical isolates were correlated to a low prevalence of gentamicin resistance (de FATIMA SILVA LOPES et al. (2005) in WÖGERBAUER, 2007). A decrease in kanamycin resistant *E. coli* isolates was noted in the UK (BEAN et al. (2005) in WÖGERBAUER, 2007). From 254 *C. jejuni* isolates, 8 were resistant to kanamycin. Molecular analysis showed the aminoglycoside phosphotransferase aphA-3 to be responsible for this phenotype (GIBREEL et al. (2004) in WÖGERBAUER, 2007).

Resistance levels to neomycin were reported to be low in *E. coli* isolates from pigs (BRUN et al. (2002) in WÖGERBAUER, 2007). TRAVIS et al. reported kanamycin resistance rates of 56%, 15% and 10% in enterotoxigenic *E. coli*, non-enterotoxigenic *E. coli* and in commensal *E. coli* isolates from pigs, respectively (TRAVIS et al. (2006) in WÖGERBAUER, 2007). In poultry litter no coliform isolate was resistant to kanamycin or neomycin. 5% of the isolated pseudomonades were resistant to kanamycin and 10% of them were resistant to neomycin, which was usually part of a multi-resistance locus (KELLEY et al. (1998) in WÖGERBAUER, 2007). Stability of neomycin resistance may be variable in the rhizosphere (HALVERSON et al. (1993) in WÖGERBAUER, 2007).

In conclusion all these data indicate a high variability of the incidence of resistance to neomycin and kanamycin antibiotics. There are habitats and ecological niches with an extremely low resistance rate as well as environments which favour a higher prevalence of resistance presumably reflecting altering effects of a variable selection pressure. Different bacterial species may differ substantially in the susceptibility to these antibiotics (WÖGERBAUER, 2007). That the occurrence of resistance to neomycin and kanamycin varies substantially between countries and bacterial species, is also concluded by EMEA (2007).

Therefore maintaining the indifferent opinion of a generally high background load of resistance to kanamycin or neomycin is not based on scientific facts and cannot be regarded as appropriate.

The EFSA GMO Panel maintained in its opinion (2007b) that aminoglycoside resistance determinants are highly prevalent in natural environments and referred to references from 1993 (SMALLA et al. (1993) in WÖGERBAUER, 2007); moreover, nptII is usually located on a transposon, and thus, easily spread by horizontal gene transfer (BLAZQUEZ et al. (1996), KOTRA et al. (2000), NORMARK B.H. and NORMARK S. (2002) in WÖGERBAUER, 2007). Therefore EFSA (2004b) concluded that the contribution of transgenic ARM gene derived nptII sequences to the global resistance gene pool is negligible. But this conclusion is neither backed by a permanent analysis of the resistance status of certain reference strains, which nowadays are successfully used for the monitoring of the dissemination of antibiotic resistance in Germany and Europe (GENARS, EARSS), nor did EFSA acknowledge differences in the background level of resistance between various European countries (WÖGERBAUER, 2007).

It has to be stated, that for a serious risk assessment the local resistance profiles of certain bacterial monitoring strains have to be collected. The actually appearing level of resistance was exemplified with five representative pathogenic

bacterial strains (*Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae* as described in WÖGERBAUER (2007): The chosen pathogens are reference strains in large national and international networks (EARSS, GENARS) which have been installed to monitor antibiotic resistance in clinically relevant pathogens (GRUNDMANN et al. (2004), HUPPERTZ et al. (2004) in WÖGERBAUER, 2007). All these bacteria can cause severe, life threatening, infectious diseases including hardly treatable hospital-acquired infections:

- Resistance rates to a single antibiotic class are widely varying between different pathogenic species. The phrasing of a generally valid statement is impossible.
- For the discussion of antibiotic resistance background levels it is imperative to agree upon which strains to be used for assessment.
- An indifferent statement about generally high (or low) background levels of resistance in bacteria is impermissible. Actually observable resistance rates show a meaningless range from 0–100%, according to the particular strain under observation.

These local peculiarities have to be taken into consideration for a risk assessment of the ARM gene under scrutiny.

A risk assessment for nptII should also consider the possibility of mutational alterations of ARM nptII gene sequences, possibly altering the spectrum of inactivated antibiotics. A single mutation in nptII might lead to the phosphorylation of amikacin (KOCABIYIK and PERLIN (1992) in WÖGERBAUER, 2007). However, PERLIN et al. showed that for induction of a high level resistance to amikacin at least two simultaneously occurring mutations were necessary (PERLIN and LERNER (1986) in WÖGERBAUER, 2007).

In a rumen microbial ecosystem nptII gene transfer was observed without any kanamycin selective pressure (MALIK et al. (2003) in WÖGERBAUER, 2007). An interesting aspect would also be the potential of nptII to inactivate future, less toxic, aminoglycosides (WÖGERBAUER, 2007).

It should also be mentioned that an integration of nptII into the plastid genome leads to an enormous amplification of the nptII gene (more than 10 000 copies) per plant cell, compared to less than 10 copies if the insertion site is nuclear (CARRER et al. (1993) in WÖGERBAUER, 2007).

#### **4.4. Selective conditions promoting survival of nptII carrying bacteria**

The dissemination of antibiotic resistant bacterial strains in a clinical setting is not predominantly the direct result of initially high transfer frequencies for resistance genes, but more often clinical problems arise only due to the selective amplification of bacterial cells carrying resistance determinants. A positive selection is usually necessary to outnumber and establish larger bacterial populations (NIELSEN and TOWNSEND (2004) in WÖGERBAUER, 2007). Information on the usage pattern of commercially available antibiotics allows the identification of environments where strong positive selection for bacteria carrying the appropriate resistance function is likely to occur (WÖGERBAUER, 2007).

For example: In Norway 10 g neomycin were used for human therapeutic applications in 2004, whereas 31 kg were applied for veterinary purposes (NIELSEN et al. (2005) in WÖGERBAUER, 2007). 30 tons of neomycin and kanamycin are used annually in Dutch agriculture (NAP et al. (1992) in WÖGERBAUER, 2007). In Denmark 11 tons of aminoglycosides are used in the veterinary sector (DANMAP, 2004) in WÖGERBAUER, 2007). These data illustrate a completely divergent usage pattern of antimicrobials throughout Europe, creating environments with completely different selection pressures (CARS et al. (2001) in WÖGERBAUER, 2007). It is noteworthy that the analysis of NAP et al. does not exclude aminoglycoside levels in soils, which would provide a selective pressure for nptII propagation (NAP et al. (1992) in WÖGERBAUER, 2007). Antibiotics may accumulate on agriculturally used fields and in soil environments providing selective conditions (BLACKWELL et al. (2007) in WÖGERBAUER, 2007).

#### **4.5. Probability of gene transfer from the GM plant to bacteria**

The statement of EFSA that horizontal gene transfer from transgenic plants to competent recipient bacteria is an extremely rare event - especially in natural habitats - is basically correct.

Whereas the notifier (GAY, submitted 2003) stated that “no evidence of a successful de novo transfer of a functional ARM gene from genetically modified plants to bacteria in natural environments has ever been reported...”.

But transfer of nptII from plants to bacteria has already been shown and published by some research groups (e.g. TEPFER et al. (2003)) as indicated already by EMEA (2007), though EFSA (2007a) is arguing that gene transfer from plants to bacteria has only been demonstrated under laboratory conditions when regions of homology were already present in the recipient bacterium”.

However, concerning the risk assessment of ARM genes the frequency of gene transfer events as argument for a low immanent risk is not cogent (NIELSEN et al. (2005), PETTERSEN et al. (2005) in WÖGERBAUER, 2007) ). This may be exemplified by following consideration: A single transfer event of an ARM gene from a transgenic plant to a bacterium may be enough to establish a resistant founder generation of microbes which may later on interfere with antimicrobial therapy directly or by passing on the resistance function to human pathogens. The establishment of such a founder generation may take long periods and may remain undetectable for some time (HEINEMANN and TRAAVIK (2004) in WÖGERBAUER, 2007).

Furthermore there are also indications that horizontal gene transfer processes in natural environments are not recorded properly using the currently available methodology (NIELSEN (2003), NIELSEN and TOWNSEND (2004), PETTERSEN et al. (2005) in WÖGERBAUER, 2007).

#### **4.6. nptII as selectable marker system: regulatory and market acceptance**

A vast array of transgenic plants relying on this marker system has been approved for marketing in several countries. The EFSA GMO Panel pointed out that for group I ARM genes there is a 13-year history of safe use of transgenic plants (EFSA, 2004b), but this statement is not backed up by any controlled scientific study.

During this 13-year period of safe use - especially in the United States - (expanded by Goldstein et al. to 15 years (GOLDSTEIN et al. (2005) in WÖGERBAUER, 2007), neither one has systematically looked after any side effects among the American population, nor has any monitoring program been active. In addition to this, no attempts have been made to specify the term "safe use" (WÖGERBAUER, 2007).

However, the presence of the superfluous nptII gene in the adult transgenic plant exacerbates the risk assessment, and the presence of antibiotic resistance genes increases public and consumer scepticism (WÖGERBAUER, 2007).

#### 4.7. General Conclusions

1) The statement that most of the antibiotics inactivated by ARM genes are outdated and no longer in use does not take into account the actual therapeutic relevance of kanamycin, neomycin and paromomycin in human and veterinary medicine as well as the remarkable differences in antibiotic consumption and prescription patterns between different countries of the European Union. This diverging antibiotic usage leads to completely different resistance profiles of clinical isolates in each of these countries. These local peculiarities for the application of antimicrobials have to be taken into consideration for a risk assessment of the ARM gene under scrutiny.

Furthermore aminoglycosides as a class have become more and more important as alternative treatment options due to the rise of multi-resistant bacterial strains (e.g. *M. tuberculosis*). Kanamycin and neomycin have been classified as critically important antibiotic by a WHO working group (2005).

2) The most prominent argument for an unrestricted use of ARM genes in transgenic plants is the marginal low frequency of gene transfer of ARM genes from the plant genome to competent bacteria in natural environments. EFSA argued that a low gene transfer frequency in natural habitats is equivalent to a low risk for adverse effects. Unfortunately, frequencies are of little predictive value in the assessment of long-term effects of sporadic gene transfer events, particularly because the relevant transfer frequencies may well be below current methodological detection thresholds. A single successful ARM gene uptake event may be sufficient to build a founder generation for a subsequently resistant bacterial strain.

3) With regard to the kanamycin and neomycin resistance in natural environments it has to be stated that the components of the global pool of resistance genes are in a constant flow, reacting to continually changing environmental conditions. Genetic information is constantly exchanged between the participants of the microbial ecosystem. Substantially different country-specific application patterns for antimicrobials are not taken into consideration and additionally the analysis does not consider the highly dynamic nature of resistance in natural environments, where resistance genes are readily exchanged via horizontal gene transfer adapting to the relevant selection pressure prevailing in the habitat.

A generalizing statement about the status of resistance in natural environments is unreliable.

4) Indiscriminative high background resistance levels in naturally occurring bacterial populations are postulated: This statement communicates the impression of a low risk process if ARM genes additionally are introduced into an ecosystem. But this proposition does neither take into account strain- and species-specific differences in resistance levels, nor does it acknowledge locus-, habitat- and country-specific differences in the resistance rates of the same bacterial species. The actually occurring resistance status was verified with kanamycin and neomycin (nptII). This example provides evidence for a low prevalence of both resistance functions in many environments and strains analyzed.

5) In the EFSA risk assessment any quantitative data concerning the copy number of resistance determinants in receptor populations (background-level of resistance) or the potential input copy number of ARM genes via transgenic organisms is missing. But a quantitative understanding of this phenomenon is necessary for a serious assessment of the effect of additionally introduced ARM genes.

6) Recent data have indicated the presence of considerable amounts and persistence of various antibiotics in soil and on, and even in, plants from manure of animal husbandry. The application of antibiotics for the treatment of infectious diseases in animals also provides substantial stress on bacterial populations. Therefore positive selection pressure even in field environments is not unlikely.

In accordance with the ad hoc group of the Norwegian Scientific Panel on Genetically Modified Organisms and the Panel of Biohazards, significant and distinct differences in antibiotic usage levels and antibacterial resistance rates between European countries could be identified. This observation implies the necessity of a case-by-case risk evaluation of each notification taking into account country-specific peculiarities. A large-scale introduction of ARM genes via transgenic crop plants leads to a different outcome of the risk assessment in areas with a low incidence of the equivalent resistance functions compared to environments with an intrinsic high background level of resistance. This consideration is also relevant for the group 1 ARM gene nptII (WÖGERBAUER, 2007).

#### **4.8. Recommendations**

1) Due to the lack of available convincing, quantitative data concerning the nptII gene and its deliberate release, respectively the placing on the market, risk research done by independent scientists should be carried out.

2) For monitoring of antibiotic resistance gene background load certain bacterial reference strains should be chosen.

3) Before introducing genetically modified products containing ARM-genes, special care should be taken in countries with a low incidence of aminoglycoside resistant pathogens.

A massive dissemination of nptII containing DNA fragments via transgenic crop plants will certainly lead to alterations in the exposure locus and exposure rate of soil and gut bacteria not previously available for these bacteria.

4) Due to the temporal and local restrictions of field experiments the opportunity for efficient gene transfer from GMOs to bacteria may be limited, but a contrary situation is given if transgenic crops are commercialized, exposing the bacterial communities for decades and over large areas to ARM-genes.

Therefore it is highly recommended - and also laid down in Directive 2001/18/EC 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 ARMs in GMOs which may have adverse effects on human health and the environment". As a consequence a reduction of the artificial ARM gene exposure level to bacterial communities to a minimum should be made.

5) All these scientific facts underline the Austrian position that a comprehensive risk assessment of the nptII gene including the evaluation of potential long-term and indirect effects has to be carried out before the placing on the market of products containing this ARM-gene. Therefore a reanalysis of the safety assessment of the nptII gene taking into account the above mentioned criteria by independent scientists is highly recommended.

## 5. Accidental spillage and risk management measures

According to Annex VII of Directive 2001/18/EC a monitoring plan, which should cover surveillance for unanticipated adverse effects must be included. The objectives of the monitoring plan are to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the environmental risk assessment.

Accidental spillage presents one way of unintended release of maize MON 863 into the environment. In addition, exposure of the environment to the Bt toxin expressed in MON 863 (Cry3Bb1) is possible via the consumption and excretion of maize MON 863 when used as animal feed.

The monitoring plan submitted for maize MON 863 does not foresee monitoring of accidental spillage and possible ecological consequences arising from accidental spillage or other forms of introduction of the transgene products in the environment. In addition, no specific risk management strategies in case of unintentional release are provided. The notifier has missed to establish surveillance or management systems which are suitable to monitor and detect possible unintended environmental exposure by accidental spillage or release of MON 863 nor has the notifier shown that measures were taken that ensure that the reporting of unintended environmental release will be carried out by the relevant stakeholders involved. The specific monitoring methodology must include information on the methods, the frequency, and the intervals of monitoring actions carried out by the selected networks. This comprises the monitoring along transportation routes, ports and harbours, processing plants etc. An active monitoring of not only substantial but also small grain losses at diverse locations including an analysis of potential areas of concern and exposure pathways should be performed. Environmental institutions have not been involved to cover potential unexpected effects derived from accidental spillage or exposure of the environment by the use of maize MON 863. These organisations shall be involved in the monitoring network to actively take part in the monitoring and to assist the notifier in the monitoring.

In conclusion the applicant's proposal for an environmental monitoring plan does not meet the objectives defined in Annex VII of Directive 2001/18/EC and the supplementing guidance notes (Council Decision 2002/811/EC).

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