HUNGARIAN MORATORIUM

for the permission for the application for the placing on the market of the potato EH92-527-1, (Unique identifier BPS-25271-9) for food and feed uses and cultivation of potato EH92-527-1 for the production of starch

On 2 March 2010 the European Commission adopted two decisions regarding the Genetically Modified Amflora potato: authorised the cultivation of a GM potato (Amflora) in the EU for industrial use (starch production and use in the paper industry), and allowed the use of Amflora's starch by-products as food and feed within the EU. The authorisations are valid for 10 years.

Unfortunately, according to EU regulation, there is no way to release a GMO/LMO for feed use only, the law certifies the release for food and feed at the same time. Therefore, release as feed also allows the entry of AMFLORA potato to the food market.

This potato, the EH92-527-1 is derived from the cultivar Prevalent. Potato leaf discs were transformed by Agrobacterium-mediated gene transfer technology. The modification involved inhibition of the expression of granule bound starch synthase protein (GBSS) responsible for amylose biosynthesis. A gene conferring kanamycin resistance (nptII) was used as a selectable marker. Molecular analysis showed that potato EH92-527-1 contains two partial copies of the DNA fragment, the insert, including the flanking region, was duplicated in reverse orientation and joined tail-to-tail. This is present at a single locus in the nuclear genome of the GM plant.

The certificarion of the AFLORA potato has a long history.
- The Amflora approval process started with a request for authorization in 1996. The scope of the application included cultivation, industrial use and the use of the pulp as feed.
- BASF Plant Science resubmitted a dossier for cultivation in 2003, and another dossier for food and feed use in 2005.
- In 2006, the EU Commission published two EFSA assessments, stating that Amflora is as safe as the conventional potatoe as food and feed, and it is also safe for the environment.
- In 2006 Amflora was put to the Regulatory Committee, consisting of all EU Member States. After two inconclusive votes in 2006 and 2007, the EU legislation did not adopt the proposal for cultivation.
- The dossier for food and feed use was voted upon by the Standing Committee consisting of members from all EU Member States in 2007 and by the Council of Agricultural Ministers in 2008. Since the National governments could not reach a decisive opinion on whether to approve or reject the potato variety, neither when they voted on the issue in an expert committee nor when the issue was put to the Ministers, the decision was passed on to the EU Commission, who requested EFSA to prepare an opinion on the use of antibiotic resistance marker genes in genetically modified plants (EFSA (2007). Statement of the GMO Panel on the Safe Use of the nptII Antibiotic Resistance Marker Gene in GM crops (Genetically Modified Plants. European Food Safety Authority. <http://www.efsa.europa.eu/en/science/gmo/statements0/npt2.html%20>http://www.efsa.europa.eu/en/science/gmo/statements0/npt2.html .) Such a marker gene is used in Amflora. It was stated that the EU Commission will give permission only if EFSA confirms the safety of Amflora.
- In 2008 after the vote in the Council of Agricultural Ministers (the last formal step prior to a decision) BASF Plant Science filed an action with the European Court of First Instance against the EU Commission for failure to act.
- In 2009 EFSA published its final, positive opinion on the use of antibiotic resistance marker genes in genetically modified plants, paving the way to release Amflora (EFSA, 2009: Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants.

<http://www.efsa.europa.eu/en/scdocs/doc/1108.pdf>http://www.efsa.europa.eu/en/scdocs/doc/1108.pdf).
- Based on EFSA's positive opinion, in 2010 the European Commission approved both the commercial cultivation and the food and feed use of Amflora potatoes in Europe.

According to Health and Consumer Policy Commissioner John Dalli "all scientific issues, particularly those concering safety, have been fully addressed".

However, it is the opinion of the Hungarian Authorities, that several questions have not been fully answered by the Dossiers and several problems have not been studied to satisfy safety concerns.

HEALTH CONCERNS

During the authorization process, one major sticking point was and still is

1. The use of the antibiotic marker gene(s).

The reason for this was stated in the Executive Summary of the Risk Assessment of Antibiotic Resistance Marker Genes in Genetically Modified Organisms (2007) by Markus Wögerbauer, commissioned by the Austrian authorities (Wögerbauer M., 2007: Risk Assessment of Antibiotic Resistance Marker Genes in Genetically Modified Organisms, BMGFJ - Forschungsberichte der Sektion IV, Band 5/2007.
<http://www.bmgfj.gv.at/cms/site/attachments/7/4/2/CH0810/CMS1198058130229/woegerbauer_2007.pdf>http://www.bmgfj.gv.at/cms/site/attachments/7/4/2/CH0810/CMS1198058130229/woegerbauer_2007.pdf)

This report states that certain antibiotic resistance marker genes present in genetically modified organisms inactivate antibiotics which are in clinical or veterinary use. Therefore concerns have been raised that the large-scale release of these antibiotic resistance marker genes by marketing or deliberate release of transgenic organisms in field trials will increase the rate of antibiotic resistant bacteria leading to reduced therapeutic options for the treatment of infectious diseases.

These concerns are habitually dismissed based on the claim that resistance to such antibiotics is already common in soil bacteria. However, that argument lacks merit in the case of a safety assessment because the kinds of bacteria that cause disease in people and animals are not soil bacteria and the resistance is not, or previously has not been, common in these pathogens (Heinemann, 1999, Heinemann et al, 2000). Moreover, there are no studies that can dismiss the potential for increased transfer of resistance genes from ingested plant material and commensal or pathogenic bacteria in the human or animal gut. However, it is clear that the human gut is condusive to gene transfer between bacteria and from bacteria on ingested food to gut bacteria (Ferguson et al., 2002, Hehemann et al.,2010). It is no longer acceptable to ignore the importance of using resistance genes in our food.

This problem was also acknowledged by the authorities of the European Parliament and the European Commission and was included into the Directive 2001/18/EC, which requires a step-by-step phasing out of ARM genes in GMOs, which may have adverse effects on human health and the environment (Art. 4 (2) Dir. 2001/18/EC) by the end of 2004 (concerning GMOs released for market according to part C) and 2008 (concerning GMOs authorized according to part B) respectively.

By stating that the ARM gene is safe in the Amflora potato, EFSA has over-written the EC regulation and overruled its prior opinion from 2004 without any new data on the probability of horizontal gene transfer. To do this EFSA experts consider that a general ban on these markers is not justified, recommending instead a "differentiated and sensible approach": some antibiotic-resistance markers are to have their approval withdrawn, others are to be authorised only with restrictions. They concluded that "Nothing will change as far as the nptII marker gene

is concerned" - a gene that has been used in most GM plants and confers resistance to the antibiotic kanamycin.

To justify their decision, the GMO Panel of the EFSA classified the antibiotic resistance genes as markers into three groups:

Group 1: ABR genes which are already widely distributed among naturally occurring micro-organisms. The antibiotics concerned are of no or only very limited relevance for human and animal medicine. This group includes the nptII gene (kanamycin resistance, the conventionally and commercially most frequently used selection system)) and the hph gene (hygromycin). the conventionally and commercially most frequently used selection system based on nptII.

Example: nptII gene. This marker gene, the most widely used in transgenic plants for several years, comes from a transposon (jumping gene). It confers resistance to several antibiotics including kanamycin and neomycin. However, these are only used occasionally, for instance in patients who are unable to tolerate other antibiotics. Kanamycin can also cause serious side effects. It is assumed that the use of marker genes in this group in transgenic plants will not change their existing distribution in the environment. The EFSA experts are unable to find any safety grounds to justify the restriction of these ABR genes. They therefore recommend that GM plants with these ABR marker genes should continue to be authorised without restriction for both field trials and commercial cultivation.

The ARM gene of Amflora belongs to this group. It appears taht EFSA has not changed the old classification from the time before WHO and EMEA categorised kanamycin and neomycin as highly important). With the new categorisation nptII would belong to groupII or even to group three. One might think that this grouping exists only to allow and justify the release of Amflora, and other GM plants with the npt(II)gene, as MON863!

Group II: ABR genes which are widely distributed in naturally occurring microorganisms. However, the associated antibiotics are still prescribed to control certain diseases. This applies to the ampr gene (resistance to ampicillin), the aadA gene (streptomycin) and the Cmr gene (chloramphenicol).

Example: ampr gene. This gene confers resistance to the antibiotic ampicillin. It comes from E.coli bacteria and is used in approved transgenic plants (Bt176 maize). Although ampicillin is only prescribed occasionally, it remains the agent of choice for certain infections.It is assumed that the use of marker genes in this group in transgenic plants will have virtually no effect on the existing distribution. If these marker genes were to have an impact on the health of humans or animals, it would be regarded as minimal. The EFSA experts recommend authorising the use of these ABR markers only for deliberate release trials, but not for commercially grown GM plants.

Group III: ABR genes which confer resistance to antibiotics highly relevant for human medicine.

Example: nptIII gene. This gene confers resistance to the antibiotic amikacin, an important reserve antibiotic which is effective in controlling various infectious diseases.Even if there is no proven risk of reducing the effectiveness of this antibiotic as a result of using corresponding marker genes in GM plants, these marker genes should be dispensed with as a precautionary measure. The EFSA panel advises that GM plants containing these marker genes should not be released into the environment either for trials or commercial purposes.

According to the EFSA GMO Panel, no risk is associated with group 1 resistance genes, and thus the Panel proposes that no rationale may exist to restrict or prohibit the use of Group 1 ARM genes.

Group 2 resistance genes might pose a slight risk for humans and animals, and should only be used for authorized field experiments. These ARM genes should not be used in transgenic plants that are placed on the market.

Group 3 resistance genes should be avoided in transgenic crops under all circumstances because these genes inactivate antibiotics crucial for therapy of infectious diseases in humans.

Although the EFSA contribution to risk assessment of ARM genes in plants is invaluable and impressive in the amount of data collected, some serious flaws in the data interpretation by the GMO Panel could be identified. This may in our opinion, lead to a significant bias in risk assessment of ARM genes:

As the WHO and EMEA categorised Kanamycin and neomycin as highly important), in our oppinion with the new categorisation nptII would belong to groupII or even group III.

Re-categorising npt(II) to Group III, the opinion of the Hungarian experts about this groupping and on the categorisation of the Npt(II) gene coincides with the conclusions reached in the Executive Summary of the Risk Assessment of Antibiotic Resistance Marker Genes in Genetically Modified Organisms (2007, the WHO and the EMEA.

The report comprises a risk assessment of some important antibiotic resistance marker genes based on the analysis of the relevant scientific literature available until August 2007. For an assessment of the potential risks induced by antibiotic resistance marker genes the following issues are considered:
1. Genetic and biochemical characteristics of the most commonly used antibiotic resistance marker genes (nptII, blaTEM-1, hph, aadA, nptIII, cat, tetA) and their derived proteins.
2. The prevalence of ARM gene homologues in naturally occurring microbial populations.
3. The clinical relevance of the antibiotic compounds, which are inactivated by these marker genes.
4. The frequency of the horizontal transfer of antibiotic resistance marker genes from transgenic plants to bacteria under naturally occurring conditions with a focus on DNA stability in natural environments, selection pressure, efficiency of transfer and incorporation of foreign genetic material into the bacterial genome.
5. Alternative technologies, which do not rely on the application of antibiotic resistance marker genes, are presented. Benefits, accessibility and cost efficiency are presented and compared toThe broad application of antimicrobial compounds for therapy of human infectious diseases and in animal husbandry has created by itself a large reservoir of resistance genes in naturally occurring bacterial populations. The emergence of multi-drug resistant pathogens has already severely compromised antimicrobial chemotherapy leading to a substantial increase in morbidity and mortality due to microbial infections and to a growing burden for the public health care system.

Antibiotic resistance may develop in sensitive bacteria by the acquisition of either partial or complete resistance genes via horizontal gene transfer (Heinemann and Traavik, 2004).

1. According to the Norwegian Scientific Panel on Genetically Modified Organisms and the Panel of Biohazards it is unclear how the EFSA opinion defines and distinguishes quantitatively the prevalence of the genes in relation to the group classification made. It is unclear if the prevalence argument is based on considerations of the ARM gene copy number only, or if the relative presence of ARM gene homologues among relevant clinical isolates in different countries is also considered. The lack of relevant data and quantitative definitions easily leads to subjective and contested interpretations of the relevant antibiotic usage and resistance levels for group classification.

2. The EFSA GMO Panel proposes indiscriminatively high background resistance levels in naturally occurring bacterial populations. 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/neomycin (nptII) and penicillin resistance (blaTEM-1).

These examples provide evidence for a low prevalence of both resistance functions in many environments and strains analyzed.

3. The analysis of the EFSA GMO Panel ignores substantially different country-specific application patterns for antimicrobials, and 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. This observation also includes decreasing resistance rates. The actual European usage patterns are discussed in relation to the resistance profile of clinically relevant pathogens and are exemplified with penicillin and Streptococcus pneumoniae.

4. A cornerstone of the EFSA GMO Panel's argumentation for a low risk profile, especially of group 1 and 2 ARM genes, is the argument that inactivated antibiotics are in use no longer in clinical practice. This assumption does not take into account country-specific antibiotic usage patterns. Some European countries may still rely heavily on a specific antimicrobial compound, whereas the same compound may not even be commercially available in other countries. This may be illustrated by the following examples: In Estonia, kanamycin was only recently introduced into the national TB treatment program. In Austria no kanamycin preparations for human therapeutic use are on the market. In 2004 10 g of neomycin were administered for human therapeutic applications and only 31 kg were applied for veterinary purposes in Norway. The situation was quite contrary in The Netherlands, where 30 000 kg of neomycin and kanamycin were estimated to be used in agriculture annually. In France approx. 19 daily doses (per 1000 inhabitants per day) of penicillins were administered in 2001, whereas The Netherlands consumed only 3.5 dd of those antimicrobials during the same period.

5. The EFSA GMO Panel argues 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 (this is precisely the point of (Heinemann and Traavik, 2004).A single successful ARM gene uptake event may be sufficient to build a founder generation for a subsequently resistant bacterial strain.

6. The EFSA's opinion omits 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. But a quantitative understanding of this phenomenon is necessary for a serious assessment of the effect of additionally introduced ARM genes.

7. It is stressed that rare ARM gene transformants need positive selection pressure to grow to a larger population size. Without selection pressure they will be lost. The prevalence of this kind of selection pressure in the appropriate habitats (e.g. on fields, soil etc.) is put into question by the GMO Panel. However, 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. Thus, positive selection pressure even in field environments is not unlikely. Critically, the Panel did not consider that there may be other functions to these genes in the environment and that they are also resistance genes in the presence of the antibiotic (Heinemann 1999, Heinemann et al. 2000, Heinemann, 2007)..

The EFSA GMO Panel's opinion therefore does not necessarily reflect the more precautionary motivated regulations of ARM genes for commercial use in food and feed in Austria, or in Hungary.

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.

The generally proposed high frequency of naturally occurring resistances originating from the global resistance gene pool is not uniformly reflected by the resistance profiles of clinically relevant pathogens. The most revealing example was documented by the resistance rate of Streptococcus pneumoniae to penicillins: In Germany 1-5% of all analyzed isolates were resistant to penicillin as opposed to the neighboring country France (or Spain), where up to 50% of all isolates were resistant during the same test period. An additional input of blaTEM-1-sequences from transgenic plants would meet two completely different background levels of clinically relevant resistances in these two countries. Resistance patterns between different bacterial pathogens against the same antimicrobial showed similar discrepancies: A low frequency of resistance to penicillins (< 2%) was reported for Enterococci and isolates of S. pneumoniae, whereas resistance to ampicillin was quite common in E. coli (up to 50%) and S. aureus isolates (80%).

Concerning these facts, it is not adequate to assume that a similar high background frequency of clinically relevant resistances is to be expected throughout Europe. A comparable situation was encountered for recently collected environmental samples from soil, the guts of birds, boars, pigs, cattle and poultry if analyzed for resistance to aminoglycosides. An analysis of indications for antibiotic therapies in Austria and Germany revealed that antimicrobials (with the exception of aminoglycosides) which were inactivated by antibiotic resistance marker genes still play a crucial role in anti-infective chemotherapy. Thus, it is not adequate to suggest that all inactivated group 1 and 2 relating antibiotics are "outdated" and clinically irrelevant compounds. Ampicillin, amoxicillin, and penicillin G are the drugs of choice in many cases. Even neomycin, paromomycin, and streptomycin may be a last resort for terminally ill patients or for rare exotic infectious diseases. It should also be mentioned that chloramphenicol is the drug of choice for a variety of indications in developing countries. However, hygromycin has no therapeutic application.

The nptII gene, which preferentially inactivates the aminoglycoside antibiotics kanamycin, neomycin, and paromomycin, has been classified as group I ARM gene. Due to the flaws in data interpretation (see above) and new available information, this EFSA classification might not be appropriate for all European countries: Neomycin has recently been classified as critically important antibiotic by a FAO/WHO working group. The EMEA, in accordance with the EFSA GMO Panel, has acknowledged neomycin as antimicrobial for the treatment of severe clinical conditions like hepatic encephalopathy. 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 has explicitly been recommended in the treatment of multi-resistant tuberculosis in several countries (USA, Estonia) and has been designated as potential anti-tuberculosis drug in the case of bioterrorism. An interesting long-term perspective concerning the development of novel, less toxic neomycin or kanamycin derivatives should also be considered. Their novel therapeutic option might be diminished by the effect of nptII. In veterinary medicine aminoglycosides are of crucial importance, namely neomycin, kanamycin and paromomycin, which have been explicitly licensed for the treatment of infections in food producing animals. The EMEA concludes that neomycin and kanamycin are of importance for veterinary and human use, and their current or potential future use cannot be classified as of no or only minor therapeutic relevance.

Although the risk for adverse effects on human health and environment due to the release of nptII ARM genes is generally considered to be low, 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. Due to the scarcity of available convincing, quantitative data, no recommendations concerning nptII and deliberate releases for field experiments can be given. The implicit temporal and local restrictions of field experiments might further limit the opportunity for efficient gene transfers from GMOs to bacteria, in contrast to the situation with commercialized transgenic crops, which expose the bacterial communities for decades and over large areas.

Only very limited information on hygromycin resistance background levels is available, but the classification of hph as group 1 ARM gene appears to be appropriate because hygromycin has no applications in human medicine and is only of limited use in animal husbandry.

Special precautions are recommended for the use of blaTEM-1 in transgenic organisms for commercialization and for field trials in countries with a low background level of the corresponding resistance functions in natural environments. It should be a matter of common sense to deliberately reduce the artificial ARM gene exposure level of the bacterial communities to a minimum considering the immanent clinical importance of these inactivated antimicrobial compounds (= penicillins). This should also be seen in the context of alternatives to ARM genes available. In Austria streptomycin and chloramphenicol have a narrow range of indications and are administered rarely. Nevertheless, these cases are usually due to life threatening infections with no other treatment option left. Therefore, therapeutic importance is significant for certain patients. The background resistance levels may vary similar to those of as blaTEM-1 and nptII. Approval for them being used as ARM gene for deliberate releases in field experiments will depend on countryspecific resistance levels to streptomycin and chloramphenicol and the relative importance of these antimicrobial compounds in human medicine and veterinary applications. The classification of nptIII and tetA as group 3 ARM genes is supported by scientific evidence, and therefore, appropriate.

In conclusion, we recommend accepting the ARM gene classification of the EFSA GMO Panel as valuable basic framework. However, due to substantial knowledge gaps, local differences in resistance levels and antibiotic usage patterns, and an overall scarcity of quantitative data concerning ARM gene copy number input and the copy number of preexisting similar resistance functions in naturally occurring bacterial populations, it should be the responsibility of the respective Competent Authority to evaluate the risk in a case-by-case approach. This procedure implies that the National Competent Authority ought to have the opportunity to re-classify the ARM gene under consideration of local prevailing preconditions. An agreement about the use of distinct bacterial indicator strains for the monitoring of ARM gene specific antibiotic resistances on a European scale would be certainly helpful under theses circumstances. However, the final decision about approval must be based on scientifically collected national datasets and must be open to scrutiny.

There are serious concerns about the npt(II) gene, including one recognised by the World Health Organisation. They consider kanamycin essential to medicine, for example in the treatment of tuberculosis (WHO, 2007:. Critically important antibacterials for human medicine for risk management - Categorization for the Development of Risk Management Strategies to contain Antimicrobial Resistance due to Non-Human Antimicrobial Use, Department of food safety, zoonoses and foodborne diseases: 34. <http://www.who.int/foodborne_disease/resistance/antimicrobials_human.pdf>http://www.who.int/foodborne_disease/resistance/antimicrobials_human.pdf).

The European Medicines Agency (EMEA) confirms that antibiotics in the kanamycin group play an important role in treating certain infectious diseases in human and animal medicine, and kanamycin is an important reserve antibiotic for the treatment of tuberculosis (EMEA (2007). Presence of the antibiotic resistance marker gene nptII in GM plants for food and feed uses. EMEA/CVMP/56937/2007

<http://www.emea.europa.eu/pdfs/human/opiniongen/5693707en.pdf>http://www.emea.europa.eu/pdfs/human/opiniongen/5693707en.pdf).

Even two members of the BIOHAZ Panel expressed minority opinions. One of their concerns is that if a horizontal gene transfer event were to take place despite the extremely low probability, any adverse impacts on the environment and human health cannot be adequately assessed (EFSA, 2009: Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants <http://www.efsa.europa.eu/en/scdocs/doc/1108.pdf>http://www.efsa.europa.eu/en/scdocs/doc/1108.pdf).

2. The jumping genes destabilize the genome and help horizontal gene transfer.

The nptII gene, this marker gene is the most widely used in transgenic plants for several years, comes from a transposon (jumping gene). In traditional plants normally transposons never leave the cells they are present in.

Using "mobile genetic elements" - in this case a plasmid, a small DNA unit which is independent of the main chromosome - makes it easy for bacteria can exchange genetic material very easily on a large scale. This makes possible the gene flow for ntp(II). But a plasmid is not used in the plant.

Vertical gene flow: Plant to plant gene transfer

Potato is mostly propagated vegetatively with tubers, but certain varieties can also come from seeds. Pollen can spread a certain distance. According even to EFSA, the genetically modified potato can spread its genes to other cultivated potatoes, although it is stated that EH92-527-1 and the mother clone Prevalent abort most flowers prematurely, and the stamens produces almoust no pollen. But seed plants and plants from tubers and other vegetative parts that have been left behind during cultivation are not always destroyed by tilling or by chemical weed management. Even EFSA acknowledges that natural exchange of genetic material is possible with other varieties of potato, Solanumtuberosum. The genetic spread is assessed as limited to crosspollination with other potatoes, although the chance of any transfer is considered to be low, but it is still there.

Horizontal gene flow: Plant to bacteria gene transfer

Resistance genes in bacteria are often located on mobile DNA units, which can be exchanged between different species. Based on our calculations horizontal gene transfere occurs very often indeed (Heinemann and Traavik, 2004).

If genes expressing resistance to antibiotics are spread to pathogenic strains of bacteria, infections caused by such bacteria would not be treatable by the antibiotics in question.

Bacteria are capable of absorbing plants genes and are capable of exchanging genetic material directly between each other - and even across species boundaries. Bacteria, although in very small quantities, are also able to absorb plant DNA, and hence could also exploit those genes which have been newly inserted into genetically modified plants,as it was eviodent from the Newcastle experiment (Netherwood, 2004). As the Amflora potato, although it is intended exclusively for the starch industry, has also been approved by the European Commission for food and feed, the waste material (pulp) from the starch production will be used as animal feed. Since the transgenes cannot be removed from the pulp, the chances of gene transfere of the npt(II) gene between plant transgene and the gut bacteria of animals consuming the palp are very likely. The modified potato contains an nptII gene for kanamycin resistance with the potential for transfer from plant material into microbes in the gastrointestinal tract of animals

(Chowdhury et. al., 2003), or to bacteria in the soil (specially, if the starch processing by-products, fruit juice, would be placed on the market to be used as any conventional starch potato by-products as fertilizer, contaning the transgenic DNA is a freely available form).

Also, the transgenes can be secreted by the plant roots into the soil during plant life, or from decomposting plant parts. According to general observation of the potato growers, during and after cultivation there are always live plant cells and plant parts remaining in the soil, making gene transfere even more easily possible.

Considering that we do not know much about 90% of soil bacteria, their genetic composition, and how their genes can be passed on by one strain to another for example, by connugation, we cannot assess the risks of horizontal gene transfer properly. Specially, if the transfer occurs between the GM plant or other bacteria, than passed on to a patogenic bacteria!

Since the pulp is produced in high speed rasping machines, the potato cells are broken open and their transgenic DNA is freed and easily available, therefore the chances of horisontal gene transfere into the gut bacteria of ruminants is higher than in case of feeding whole plant cells as feed.

3. One resistance brings on the next.

Bacteria can exchange genes within and between species by different biochemical pathways, such as conjugation, transformation and transduction. Since resistance genes spred,antibiotic resistance can occur easily. It is also well known in the scientific literature than after acquiring resistance to one antibiotic, the same bacteria may pick up other resistance genes much easier and become resistant to other antibiotics in a much shorter time. When antibiotics are present in the environment, because of positive pressure for selection, antibiotic resistance is a competitive advantage (Heinemann,1999, Heinemann et al., 2000).

4. The use of antisense strategy

Using the antisense strategy, they switched off the gene for the starch synthase enzyme, which is involved in the synthesis of amylase. However, antisence technology produces double stranded RNAs. The dicer might cut these sequences in to siRNA or riRNAs. These pieces of RNAs might turn off other genes in addition to the target gene.

dsRNA: RNA is synthesized through the reaction called transcription as a single strand, but may fold to create intra-molecular base pairs or may hybridize through base-pairing with a molecule of complementary sequence. When this happens, dsRNA is created. dsRNA may be processed into forms with gene regulatory activity and revealed through a number of phenotypes such as RNAi (RNA interference), PTGS (post-transcriptional gene silencing) and others. The small dsRNAs are called si (short-interfering)RNA, mi (micro)RNA, sh (short hairpin) RNA and by other names.In the dossiers there is no data on these. The primary risk issues concerning dsRNAs is that as a result of the engineering process or the rDNA itself:
- novel small and possibly dsRNAs have been created in the GMO;
- endogenouos dsRNAs are being expressed de novo or at new concentrations that are now biologically relevant.
- Either in the GMO or
- In animals or humans that may consume the GMO.

A combination of in silico and experimental techniques are available to survey both endogenous and novel small RNA molecules that might become part of the dsRNA pathways that regulate gene expression and which may also cause heritable effects.

The most comprehensive experimental tool is high throughput sequencing. Microarrays can also be used, but they are less likely to survey all small RNA molecules. These tools can also be used to determine if endogenous dsRNAs are being expressed to different concentrations in the GMO as compared to the conventional counterpart.

In silico techniques should be used to focus a search for novel dsRNA molecules in the GMO. This requires listing all intended new RNA molecules and all unintended but potential RNA molecules that might arise from transcription in or near the indel. For this exercise, the potential transcripts made from both strands of DNA should be used, and should include upstream and downstream sequences, to capture alternative promoters, as well as introns (Ying, S.-Y. & Lin, S.-L,, 2004).

The only way to detect unanticipated novel RNA molecules is to profile the transcriptome, preferably using high throughput sequencing. Completing this analysis will provide the developer with a full list of anticipated, unintended and unanticipated RNA molecules as well as unanticipated and unintended changes to the concentration of endogenous dsRNA molecules.

These RNA molecules should be analyzed for the potential to form dsRNA either through intramolecular base-pairing (stem loop structures) or intermolecular pairing (e.g. using techniques developed by Hofacker, I. L. et al., 2004; Lyngso, R. B. et al., 1999; Reeder, J. et al., 2006; Steffen, P. et al., 2006). Then, RNA predicted to contain secondary structures should be examined as potential regulatory molecules. There are some web-based tools for this (e.g. references in Vareková, R.S. et al., 2008), but the user should be aware that most tools exist to create dsRNA for researchers rather than serve to predict possible dsRNAs. The developer should then report on any genes that might be targets of these dsRNAs in the GMO and in any other organism of interest that might eat the GMO.

For example, if the GMO is being considered for use in human food, the human genome should be challenged with the potential dsRNAs in the GMO to see if there are any potential targets in humans. This analysis should also be done using experimental techniques and animal studies . For example, the developer should report on results of microarray or high throughput sequencing analyses using the mouse genome and RNA extracts from the intestinal cells of mice fed the GMO and specific species of dsRNA unique to the GMO.

5. Compositional assessment

Comprehensive compositional analsyis were performed for amylopectin potato event EH92-527-1. The analysed compounds included dry matter, protein, fat, ash, carbohydrates, fibre, digestible fibre, fructose, glucose, sucrose, starch, chlorogenic acid, solanine, chaconine, nitrate, vitamin C, and minerals (Na, K, Ca, Mg, P, Fe, Zn, Cu, Mn, Cd). In addition to the intended alterations in starch composition, several statistically significant differences were observed between the GM potato and its control: a decrease in dry matter content, an increase in sucrose content (1.7g/100g vs. 1.2g/100g) , in vitamin C content (67 mg/100g vs 49 mg/100g) respectively, and also a decrease in total glycoalkaloids (relative to Prevalent). Although these statistically significant differences were considerted by BASF Plant Sciences as an advantage rather than a drawback, but the existence of these significant differences clearly indicate that in fact the GM and the parent potatoes are not substiantially equvivalent.

6. Nutritional assessment of GM food/feed

According to EFSA, the amylopectin potato EH92-527-1 is compositionally and nutritionally equivalent to conventional starch potatoes except for the introduced traits. The conclusion by the authorities that amylopectin potato clone EH92-527-1 is as nutritious and safe as conventional starch potatoes when its products are used in animal feed applications as any other starch potato products means that no nutritional tests were performed. EFSA authorised the unintended presence of GM potato tubers in other potatoes with a maximum level of 0.9%. The Hungarian authorities, based on the lack of substantial equvivalence (see above) cannot agree to allow the use of this GM potato as food or feed, and cannot allow the contamination of the food chain even at the level of 0.9% set by EFSA as safe. Substancial equvivalence is not a scientific term or argument either, when it comes to diseases (just think of the chemical composition of healthy and BSE infected cows!).

Although EH92-527-1 is not intended for food, the application and approval under Directive 2001/18/EC does include food use. However, no nutritional evaluation has been performed. Therefore it is very important that the potato is kept out of the food chain. Although the Notifier thought that it has established a far-reaching quality assurance system for keeping EH92-527-1 separate from other potatoes, but contaminations cannot be completely ruled out. Accidents have happened and will continoue to happen. Therefore, proper nutritional tests should have been performed, especially that the EU permission does not certifies any products as feed use only!

Special problems can be expected with diabetics, since Amflora starch rich in amylopectin, which is digested quicker than amyloseand having a higher GI index gives than starch from the parent potato. This exposes sensitive individuals to a swifter increase in blood glucose levels than with the intake of other potatoes. Effects on insulin have been noted in studies where animals or people for a long time ate food or feed of conventional origin with different starch composition. In Hungary unfortunately diabetes is rather common, therefore the contamination of the food chain with Amflora would be rather serious.

Anticipated intake/extent of use

The amylopectin potato clone EH92-527-1 is intended to be used as any other commercial starch potato for the industrial production of starch, although it uniquely contains the transgenic DNA and the npt(II) protein and has a much higher GI index! The starch processing by-products (fruit juice and pulp) will be placed on the market to be used as any conventional starch potato by-products as fertilizer and in animal feed, although it contains the transgenic DNA in a freely available form with increasede chances for horisontal gene transfere!

7. Toxicology

Test meals were prepared from freeze dried GM or parent potatoes in such a concentration that the potato content of the diet was 50000 ppm, or 2.5 mg/kg diet, based on OECD guidelines. GM and parent potato containing meals were administered to groups of 10 males and females Wistar rats at dietary concentration of 0 and 50000 ppm for 3 months. The substance intake on the parent potato was on avarage 3680 mg and 4335 and on the GM potato 3731 and 4374mg/kg body weigh per day by the males and females, respectively. (This concentration is near to consuming 250 to 300 g freeze dried potato per day by a 70 kg person.) According to EFSA their food consumption and weight gain was similar. There were however significant differences in organ weight and blood composition. In female animals, statistically significant differences in white blood cells and spleen weight were noted between animals that were fed the transgenic potato and those given a diet containing the parental cultivar. The findings of cysts in thyroids of males were checked by microscopy and were slightly increased on diets containing the transgenic potato compared with animals fed the standard laboratory rodent diet. Also in males the brain mass on the GM potato was significantly different (p?0.01). There was also significant differences (p?0.05) in the blood composition (MCL: 51.1±2.0 and 50.7±1.4, IWP 1.52±0.09 and 1.62±0.19). In females and the mass of the spleen and the ALP was significantly different (p?0.05, 0.58±0.14 and 0.51±0.14.)

We also would like to call attention to the fact that according to pathology reports, in the 90 days subcronic toxicological test of the 10 males on GM potato 7 had altered liver and kidney pathology and 2 had inflamedr heart. From the 10 femalesfed the GM 6 showed alterations in liver and 4 in kidneypathology. This can be a sign of a serious illneses,s pecially that Hungary leads the statistics with the so called Western diseases coused by our life style.

8. Protein degradation

Instead of the protein purified from amflora potato, the degradation of a commercially obtained preparation of purified npt(II) was studied after addition to in vitro samples of ruminal

fluid obtained from fistulated sheep (Fu et al., 2002). The transgenic npt(II) protein was found to be degraded to low or nondetectable levels within hours. However, this time scale is long enough to provoke allergenic reaction, which can occur within 30 mins, or sufficiently long for horizontal genetransfere to occure, since bacteria replicate in 20-30 minutes.

As far as Human and animal healths are concerned questions were raised bzy the member states regarding (1) the need for toxicological studies on the whole food/feed (e.g. 90-day subchronic oral toxicity studies in rodents exposed to whole crop or potato pulp) and the putative ORF4 protein in order to provide additional reassurance about the safety aspects of any eventual unintended effects of the genetic modification, (2) the need for additional feeding studies, including an appropriate experimental design, with other animal species, (3) the proposal for feed-testing of protein purified from potato juice on domestic animals, (4) the recommendation for extended animal feeding studies as well as genotoxicity assays, (5) the expression of the nptII gene, (6) the potential glycemic effects due to accidental human consumption of potato EH92-527-1 by diabetics and (7) the apparent increase in the number of thyroid cysts in male rats fed the GM potato) as noted in the results of microscopic examinations.

ENVIRONMENTAL CONCERNS

1. GM plant and target organisms

According to EFSA there are no specific "target organisms" for the potato EH92-527-1, and consequently no experiments were carried out with even the most common potato pests and their predators.

2. Field trials

Field trials have been carried out during various seasons in multiple locations in Sweden. Data on agronomic characteristics have been obtained from field trials carried out during 1994-1996, and variety trials during 1996-1997, while those on compositional analysis of potato tubers were from trials performed during 1996-1998. (The EFSA Journal (2006) 323, 1-20. <http://www.efsa.eu.int/>http://www.efsa.eu.int). Cultivation of starch potatoes within the European Union also occured in a selected group of Member States to which quotas for cultivation were assigned (EC, 1994). These member states were Austria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Latvia, Lithuania, Netherlands, Poland, Slovakia, Spain, and Sweden, close to industrial starch processing plants which supply the paper pulping industry. Amflora has been tested at different locations in Germany, also on a large scale. The climate, the environment, the flora and faunaofthose countries are totally different form Hungary's.

3. Coexistence: Problem secondary growth potatoes

Potatoes propagate almost exclusively vegetatively through tubers, although fertilisation of neighbouring plants through the pollen is possible.According to EFSA there are measures in place to prevent the mixing of Amflora GM potato with conventional-, or orcorganic potatoes, since the Authorising Decision contains three obligations for the consent holder to prevent the presence of the GM potato tubers in the food and feed chain. These obligations are also part of the contracts to be signed between BASF and the operators involved in the production chain (farmers and starch producers). Measures have to be taken to ensure that the potato tubers will be physically separated from potatoes for food and feed uses during planting, cultivation, harvest, transport, storage and handling in the environment,to ensure that conventional potatoes can not be planted in the same field the year following the cultivation of the GM potato; ensure that the potato tubers shall be delivered exclusively to designated starch processing plants for processing into industrial starch within a closed system. However, even EFSA acknowledges that adventitious presence can never be totally excluded, even if

EFSA authorised the unintended presence of GM potato tubers in other potatoes with a maximum level of 0.9%.

The problem for the coexistence for cultivation of conventional-, organic-, and genetically modified potatoes is primarily secondary growth. In practice it is practically impossible to remove all potatoes during the harvest. Single potatoes often remain in the soil which can germinate in the following years. As a result of human errors accidents have happened and will continue to occur.

There are also signs that the GM and conventional potatoes cannot co-exist from the field trials in Ghermany in 2007. That year, the GM potato Amflora was cultivated on nearly 150 ha in Germany. The goal of the "field trial" was to produce seed potatoest in case Amflora was allowed for commercial cultivation. It bacame obvious taht GM potatoes cannot be kept under control and they cannot be cleared off the field completely. In summer 2008, despite repeated controls Amflora potatoes were growing on the same fiels where experiments were running with amflora the year before. Anauthorised planting of Amflora has already occured in Germany near to 3 other fields where the release was approved. The illegal plantings had to be destroyed, and the authorities proposed ploughing the field - a method that left viable potatoes and potato pieces behind.

One of the conditions of the approval in 2007 was that the field would have to be controlled for volunteer plants the following year. In July 2008 Amflora plants were found both on the officially certified fields and also on the illegally planted field. The authorities ordered the destruction of the plants by herbicide, but at the second inspection in August amflora plants were still growing on the field. Some of the plants looked quite healthy, with potatoes of up to 7 or 8 cm big. However, potatoes as small a 0.5 cm can grow into new plants. During the inspection evidence of the presence of of wild boar where found on the field, and some animals were seen in the vicinity. This was a clear sign that wild animals can feed on Amflor. íthese events were not taken into consideration or into account in the monitoring plan proposed by BASF. At the time of inspection in 2007, maize was cultivated on the field the GM potatoes were grown previously therefore full visual inspection of the wholée field was impossible. To find all the GM potato plants it would have been necessary to walk through the whole field row by row.

POST-MARKET MONITORING OF GM FOOD/FEED

Questions were raised regarding (1) the need for additional information to comply with requirements of Annex VII of Dir 2001/18/EC as well as (2) the role of the Identity Preservation System in defining concrete actions, including monitoring and labelling to guarantee traceability.

The Swedish Board of Agriculture is of the opinion that the monitoring plan should also include the use of the by-product as feed. Significant deviations and any negative effects associated with the use of by-products as feed.

No post-market monitoring of GM food and feed is required at the moment, although it is obvious that this GM potato is substancially different from its parent, it is anticipated taht it would become the part of the food and feed-cahin, since from a nutritional point of view the GM potato pulp is considered as equivalent to pulp processed from conventional potatoes.

Notification C/SE/96/3501 for potato EH92-527-1 is for cultivation, and thus a monitoring plan is required that considers the environmental impact of full commercial scale, cultivation and production.

ECONOMICAL ARGUMENTS

1. Statistically significant differences between the GM potato and its control were observed each year, including a decrease in yield. It is not in the interest of farmers to grow a vareity of protato with a lower yield.
2. Hungary does not have a starch processing plant, therefore there is no need to grow the industrialal starch producing modified potatoes.

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