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SCIENTIFIC OPINION

Scientific Opinion on a request from the European Commission for the assessment of the scientific elements put forward by Luxembourg to support the prohibition for the placing on the market of GM potato EH92-527-1 for cultivation purposes in Luxembourg[1]

EFSA Panel on Genetically Modified Organisms[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Luxembourg notified to the European Commission its scientific arguments justifying the implementation of a national safeguard measure prohibiting the placing on the market of GM potato EH92-527-1 for cultivation purposes in Luxembourg, after which the European Commission asked the European Food Safety Authority (EFSA) to assess the scientific information supporting the prohibition. Having considered the information package provided by Luxembourg and all relevant scientific publications, the EFSA Panel on Genetically Modified Organisms (GMO Panel) concluded that: (i) no new data specific to the safety of the *npt*II gene have been provided; (ii) although bacterial DNA release and development of competence are expected to occur more efficiently in biofilms, the link between resistance in biofilms and cultivation/processing of GM potato EH92-527-1 was not established by Luxembourg, and the main barriers, limiting the transformation frequency of bacterial cells with transgenic plant DNA, remain; (iii) the risk posed by the formation of mosaic structures of aminoglycoside phosphotransferase genes could not be assessed without data documenting the existence of such structures among the existing gene variants, and such data were not provided; (iv) the knowledge gaps and uncertainties highlighted in the Luxembourgeois document and the therapeutic relevance of kanamycin and neomycin have already been considered in the previous EFSA opinion on antibiotic resistance marker genes, and no new information on the safety of *npt*II gene as present in the GM potato EH92-527-1 has been identified in the scientific literature that would cause the GMO Panel to change its previous conclusions. Therefore, the EFSA GMO Panel concludes that no grounds exist to date that would lead to reconsideration of its opinion on GM potato EH92-527-1.

KEY WORDS

GMOs, potato, *Solanum tuberosum*, EH92-527-1, Amflora, Luxembourg, safeguard clause, human and animal health, environment, Directive 2001/18/EC

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[1] On request from the European Commission, Question No EFSA-Q-2012-00614, adopted on 5 September 2012.

[2] Panel members: Salvatore Arpaia, Andrew Nicholas Edmund Birch, Andrew Chesson, Patrick du Jardin, Achim Gathmann, Jürgen Gropp, Lieve Herman, Hilde-Gunn Hoen-Sorteberg, Huw Jones, Jozsef Kiss, Gijs Kleter, Pagona Lagiou, Martinus Lovik, Antoine Messéan, Hanspeter Naegeli, Kaare Magne Nielsen, Jaroslava Ovesna, Joe Perry, Nils Rostoks, Christoph Tebbe. Correspondence: gmo@efsa.europa.eu

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SUMMARY

Following a request from the European Commission, the Panel on Genetically Modified Organisms (GMO) was asked to deliver a scientific opinion on the elements put forward by Luxembourg to support the prohibition for the placing on the market of GM potato EH92-527-1 for cultivation purposes in Luxembourg.

In July 2011, Luxembourg notified to the EC its scientific argumentation justifying the implementation of a national safeguard measure prohibiting the placing on the market of GM potato EH92-527-1 for cultivation purposes in Luxembourg, according to Article 23 of Directive 2001/18/EC on the deliberate release in the environment of genetically modified organisms.

On 23 May 2012, the European Food Safety Authority (EFSA) has been requested by the European Commission to assess the scientific information submitted by the Luxembourgeois Authorities in the context of a safeguard clause invoked under Article 23 of Directive 2001/18/EC.

In light of the information package provided by Luxembourg in support of its safeguard clause and, having considered all relevant scientific publications, the GMO Panel concludes that:

Luxembourg did not provide any new or additional information made available since the date of consent for this GM event that would affect the environmental risk assessment or the reassessment of existing information on the basis of new or additional scientific knowledge. New data specific to the safety of the *npt*II gene have not been provided.

Bacterial DNA release and development of competence is expected to occur more efficiently in developing biofilms than in planktonic bacterial cells. However, the link of the issue of resistance in biofilms to the cultivation/processing of GM potato EH92-527-1 was not established by Luxembourg. In addition, the main barriers, limiting the transformation frequency of bacterial cells with transgenic plant DNA, remain.

The risk posed by the formation of mosaic structures of aminoglycoside phosphotransferase genes cannot be assessed without data documenting the existence of such structures among the existing gene variants. Such data were not provided.

The knowledge gaps and uncertainties highlighted in the Luxembourgeois document and the therapeutic relevance of kanamycin and neomycin have already been considered in the previous EFSA opinion on ARM genes. EFSA continually reviews the scientific literature. No new information on the safety of *npt*II gene as present in the GM potato EH92-527-1 was identified in the scientific literature that would cause the GMO Panel to change its previous conclusions.

The EFSA GMO Panel concludes that no detailed grounds exist to date that would lead to reconsideration of its opinion on GM potato EH92-527-1.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In February 2006, the European Food Safety Authority (EFSA) adopted an opinion related to the placing on the market of GM potato EH92-527-1 for cultivation and industrial starch production, following a notification submitted by BASF Plant Science to the Swedish Authorities.

On 2 March 2010, the European Commission (EC) adopted a decision authorising the placing on the market of GM potato EH92-527-1 for cultivation and industrial starch production.

In July 2011, Luxembourg notified to the EC its scientific argumentation justifying the implementation of a national safeguard measure prohibiting the placing on the market of GM potato EH92-527-1 for cultivation purposes in Luxembourg, according to Article 23 of Directive 2001/18/EC on the deliberate release in the environment of genetically modified organisms.

In order for the EC to follow-up on this safeguard clause in accordance with Article 23 of Directive 2001/18/EC, it was deemed appropriate by EC that EFSA would assess the scientific elements provided by Luxembourg.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA was requested in accordance with Article 29 of Regulation (EC) No 178/2002 to assess the scientific information submitted by the Luxembourgeois Authorities justifying their national safeguard measure concerning GM potato EH92-527-1 and to identify whether these new scientific elements might lead the GMO Panel to reconsider its opinion on GM potato EH92-527-1 from 2006.

ASSESSMENT

1. INTRODUCTION

Directive 2001/18/EC provides the possibility for Member States to invoke safeguard measures on specific genetically modified organisms in case in which new or additional information, made available since the date of the consent, or reassessment of existing information on the basis of new or additional scientific knowledge would affect the risk assessment of an authorised genetically modified organism (GMO). Provisions foreseen by Luxembourg seek to provisionally prohibit the marketing of potato EH92-527-1 for cultivation purposes in Luxembourg.

The EFSA GMO Panel examined the set of supporting documents submitted by Luxembourg. In this respect, the GMO Panel assessed whether the submitted documents comprise new scientific information that would change the outcome of previously performed risk assessments and whether there are grounds for the GMO Panel to reconsider its opinion on GM potato EH92-527-1 (EFSA, 2006).

The EFSA GMO Panel looked for evidence for GMO-specific risks, taking into consideration the EFSA GMO Panel guidance for risk assessment of food and feed from genetically modified plants (EFSA, 2011) as well as any related risk assessments carried out in the past. In addition, the GMO Panel considered the relevance of concerns raised in the light of the most recent scientific data and relevant peer-reviewed publications regarding the use of specific antibiotic resistance genes as marker genes in GM plants.

2. CONCERNS RAISED BY LUXEMBOURG

The GMO Panel interprets the documentation provided by Luxembourg as raising the following issues:

- DNA-containing antibiotic resistance genes derived from transgenic organisms and released via plant decaying processes or ingested as food or feed would increase the likelihood of contact between antibiotic resistance-encoding DNA and competent bacteria (section 3.1);

- horizontal gene transfer by transformation occurs more frequently in biofilms, and antibiotic resistance in biofilms is of particular concern in relation to human infection (section 3.2);

- transformation of bacteria with plant-derived DNA fragments can result in the formation of mosaic genes (section 3.3);

- kanamycin and neomycin are both categorised by the World Health Organization (WHO) Expert Group on Critically Important Antimicrobials for Human Health as “Highly Important Antimicrobials” (section 3.4).

3. RISK ASSESSMENT OF THE *NPT*II GENE

3.1. The *npt*II gene as a risk factor

Luxembourg claimed that additional input of DNA-containing antibiotic resistance genes derived from transgenic organisms over extended periods of time via plant decaying processes or uptake of food or feed would increase the likelihood of contact between antibiotic resistance-encoding DNA and competent bacteria.

The GMO Panel has acknowledged this issue previously and assessed its implications (EFSA, 2009). There is no new information, either in the documentation submitted by Luxembourg or in the scientific literature that would cause the Panel to change its former conclusions. Therefore, the GMO Panel reiterates its conclusion that, taking into account all the limitations of all current methodologies of

detection, it can be assumed that there is, at most, a low probability of transfer of antibiotic resistance genes from GM plants to bacteria in the food- and feed-processing environment, in the digestive tract of humans and animals and in the wider environment. If this transfer were to occur, it would take place at an extremely low frequency. In the risk assessment, the GMO Panel took into account the subsequent development and dissemination of resistance among bacteria.

3.2. Antibiotic resistance in biofilms

Luxembourg pointed out that horizontal gene transfer by transformation occurs more frequently in biofilms and that antibiotic resistance in biofilms is of particular concern in relation to human infection. Luxembourg did not develop the relevance of the issue of resistance in biofilms to the cultivation/processing of GM potato EH92-527-1.

Antibiotic resistance in biofilms is a complex, multifactorial problem, intensively studied in relation to hospital-acquired, implant-based infections and many persistent diseases (Li et al., 2001; Beaudoin et al., 2012; Mah, 2012). Although a comprehensive understanding of this antibiotic resistance is still lacking, research results indicate the involvement of several biofilm-specific elements, not related to acquired antibiotic resistance.

Molin and Tolker-Nielsen (2003) concluded that transformation and DNA insertion by recombination in the recipient genome seem to be part of a biofilm-related life cycle. Bacterial DNA release and development of competence is expected to occur more efficiently in developing than in older biofilms. The frequency of transformation is estimated to be increased by 10- to 600-fold in competent *Streptococcus mutans* cells (Li et al., 2001).

In contrast to DNA transfer by transformation between bacteria, the main barriers in bacterial transformation with DNA from GM plants are in the uptake of DNA from decayed plant material and the absence of sequence homology between bacterial and plant DNA, limiting the stabilisation of plant DNA in the bacterial cell to the low-frequency process of illegitimate recombination (EFSA, 2009). These barriers concerning the DNA transfer from plants to bacteria, which have a high impact on transformation frequency, remain in the developing biofilms.

3.3. Formation of mosaic genes

Luxembourg indicated that transformation of bacteria with plant-derived DNA fragments can result in the formation of mosaic[4] genes. However, the Luxembourgeois Authorities did not cite any references supporting their hypothesis that *npt*II mosaic gene structures would exist with altered substrate specificity of the corresponding enzymes.

The GMO Panel is not aware of any studies reporting formation of mosaic genes between the several genes encoding aminoglycoside phosphotransferases. The Panel also notes that the example of formation of mosaic genes, as indicated by Luxembourg, was for penicillin-binding proteins, and specifically for a bacterial species in which natural genetic transformation is responsible for high genomic plasticity (*Streptococcus pneumoniae*). Extrapolation to aminoglycoside phosphotransferases is not supported by published scientific literature. Existing data do show that several conserved motifs are essential to the catalytic activity in the aminoglycoside phosphotransferase family of enzymes (reviewed by Shaw et al., 1993).

The formation of mosaic structures from the transfer of the *npt*II gene from plant to bacteria would be expected to occur with a frequency several orders of magnitude lower compared with that between bacteria. At the moment, no mosaic structures of aminoglycoside phophotransferase genes are found in bacteria although these genes are widespread in many environments.

[4] The term “mosaic” derives from the pattern of interspersed blocks of nucleotide sequence that have different evolutionary histories but are found combined in a gene allele subsequent to recombination events (Hollingshead et al, 2000).

The GMO Panel concludes that the risk posed by the formation of mosaic structures of aminoglycoside phosphotransferase genes cannot be assessed without data documenting the presence of such structures among the existing gene variants.

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

Luxembourg pointed out that kanamycin and neomycin are both categorised by the WHO Expert Group on Critically Important Antimicrobials for Human Health as "Highly Important Antimicrobials". The GMO Panel has already acknowledged this statement (EFSA, 2009; EFSA, 2012).

The GMO Panel points out that the use of antibiotics is a key factor in the selection and dissemination of antibiotic resistance genes in the immediate environment. While the key role of selection by antibiotic usage in the development of resistance seems indisputable, some knowledge gaps remain regarding the understanding of the natural reservoir of antibiotic resistance genes and their role in natural bacterial communities not exposed to industrially produced antibiotics, as concluded previously (EFSA, 2009).

In conclusion, the EFSA GMO Panel considers that the information submitted by Luxembourg in relation to the therapeutic relevance of kanamycin and neomycin was addressed in the EFSA's opinion on antibiotic resistance marker genes (EFSA, 2009) and that no additional data have been presented to warrant reconsideration.

CONCLUSIONS

The EFSA GMO Panel examined the document submitted by Luxembourg. The Panel assessed whether the document contained new scientific information and concluded that:

Luxembourg did not provide any new or additional information made available since the date of consent for this GM event that would affect the environmental risk assessment or the reassessment of existing information on the basis of new or additional scientific knowledge.[5] New data specific to the safety of the *npt*II gene have not been provided.

Bacterial DNA release and development of competence are expected to occur more efficiently in developing biofilms than in planktonic bacterial cells. However, the link of the issue of resistance in biofilms to the cultivation/processing of GM potato EH92-527-1 was not established by Luxembourg. In addition, the main barriers, limiting the transformation frequency of bacterial cells with transgenic plant DNA, remain.

The risk posed by the formation of mosaic structures of aminoglycoside phosphotransferase genes cannot be assessed without data documenting the existence of such structures among the existing gene variants. Such data were not provided.

The knowledge gaps and uncertainties highlighted in the Luxembourgeois document and the therapeutic relevance of kanamycin and neomycin have already been considered in the previous EFSA opinion on antibiotic resistance marker genes. EFSA continually reviews the scientific literature. No new information on the safety of the *npt*II gene, as present in the GM potato EH92-527-1, was identified in the scientific literature that would cause the GMO Panel to change its earlier conclusions.

The EFSA GMO Panel concludes that no detailed grounds exist to date that would lead to reconsideration of its opinion on GM potato EH92-527-1.

[5] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities L106: 1–38. (Article 23).

DOCUMENTATION PROVIDED TO EFSA

1. Letter, received 23 May 2012, with supporting document from Ladislav Miko, Deputy Director-General for the Food Chain in the EC, to Catherine Geslain-Lanéelle, Executive Director EFSA (Ref. SANCO/E1/MD/mp Ares(2012) 616525), requesting the assessment by EFSA of the scientific elements provided by Luxembourg in support of its decision to implement a national safeguard measure under Article 23 of Directive 2001/18/EC for GM potato EH92-527-1 and comprising the following supporting document:

   - Scientific justification for the ban on cultivating genetically modified potatoes (*Solanum tuberosum* L. line EH92-527-1, notification C/SE/96/3501) in the Grand Duchy of Luxembourg.

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