



**Secretariat**

O./ref.: WIV-ISP/BAC/2004\_SC\_116

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**Title: Advice of the Belgian Biosafety Council on the application Bt11 sweet maize under Regulation (EC) No. 258/97**

**Context**

On 11 February 1999, Syngenta, formerly Novartis, submitted a request under Regulation (EC) No. 258/97 to the competent authorities of The Netherlands for placing sweet corn Bt11 on the market as a novel food or as a novel food ingredient. The Netherlands' competent food assessment body came to the conclusion that Bt11 sweet maize is as safe as conventional sweet maize. Since Member States gave reasoned objections to the marketing of Bt11 sweet maize, the Commission requested the opinion of the Scientific Committee on Food<sup>1</sup>. On 17 April 2002, the Scientific Committee on Food delivered its opinion that Bt11 sweet maize is as safe for human food use as its conventional counterparts.

Sweet maize is derived from the genetically modified field maize Bt11 event through traditional breeding. Food and food ingredient products derived from the latter already have market consent in the European Union under Article 5 of the Regulation (EC) No. 258/97.

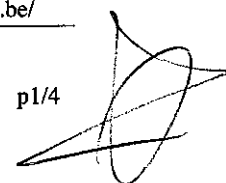
On 17 February 2003, the available molecular data of the genetically modified maize Bt11 line, including data of the notification and data provided by independent scientific bodies, were evaluated by the experts of the Biosafety Advisory Council<sup>2</sup> *a.i.*. It was concluded that the molecular data provided in the Bt11 notification did not fulfil the Belgian requirements on molecular data. The DNA sequence of the insert and the flanking regions was missing. Moreover, although no clear conclusions could be drawn from the available data, the presented data suggested that there are ambiguities concerning the molecular data of Bt11<sup>3</sup>.

In order to inform the competent authorities on the lack of essential molecular data for biosafety assessment and the possible incorrectness of the molecular data of the maize Bt11

<sup>1</sup> [http://www.europa.eu.int/comm/food/fs/sc/scf/out129\\_en.pdf](http://www.europa.eu.int/comm/food/fs/sc/scf/out129_en.pdf)

<sup>2</sup> The members of the Biosafety Advisory Council were officially nominated on 6 May , 2003. Before that date, the Service of Biosafety and Biotechnology (SBB) acted as the Biosafety Advisory Council *a.i.*.

<sup>3</sup> <http://www.biosafety.be/TP/MGC.html>



line, the report on the molecular characterisation of the Bt11 maize transformation event was sent to the Commission DG ENV, DG SANCO, competent authorities in charge of the initial assessment, on 26 May 2003.

On 17 November 2003, a response of the notifier to the Bt11 report was received by the Belgian Competent Authorities and sent to the Biosafety Advisory Council for further evaluation. The evaluation of the reply of the notifier, together with the evaluation of the Bt11 sweet maize application was put on the agenda of the meeting of the Biosafety Advisory Council of 17 December 2003. Also the reply of the notifier on the questions asked by the Belgian Competent Authorities during the consultation period (60 days period) foreseen under Directive 2001/18/EC concerning the molecular data of the Bt11 field maize were taken into account.

## Scientific evaluation

### Evaluation of reply by notifier

In its reply to the Belgian Biosafety Advisory Council, the notifier provided the sequence of the insert and the flanking DNA of the Bt11 maize event. Moreover, the notifier stated that the sequence of the insert in Bt11 is 100% identical to that of the plasmid used for transformation. In addition the sequence of the insert provided by the notifier was aligned with the one determined by an independent party. Within the insert, only a few nucleotides were found to be different between the two sequences. Such a rate of mismatch could be due to sequencing errors.

The experts of the Council concluded that *"The data presented by the Company are sufficient to lift the main ambiguities (rearrangements, truncations, insertions that have occurred), but further information should be given to lift all doubts concerning the molecular data (e.g. sequencing differences)."*

Therefore, the experts recommended (1) to check the sequence of the insert by (an) independent laborator(y)ies in order to lift the doubts concerning the correctness of the sequencing data, and (2) that a comparison of the sequencing data provided by the notifier should be made with the sequence of the vector used for obtaining the transformation event maize Bt11.

In its reply of 9 January 2004, the notifier stated that the genomic insert of Bt11 has been sequenced more than once. The alignment of the Bt11 plant sequence with the sequence of the vector used for transformation was included. The information provided by the notifier was found to be sufficient to lift the remaining doubts. Taken this information into account



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together with the information on bio-informatic analyses<sup>4</sup> provided on 3 December 2003, as requested by the Belgian Competent Authorities during the consultation period (60 days period) foreseen under Directive 2001/18/EC, it was concluded that the molecular data of Bt11 field maize fulfil the Belgian requirements concerning molecular data.

### **Evaluation of Bt11 sweet maize dossier**

The evaluation of the information in the dossier Bt11 sweet maize by the experts of the Council indicated that (1) the application presents data generated with Bt11 field maize and Bt11 sweet maize to support the food application, (2) since field maize and sweet maize are considered as safe, only the biosafety linked to the insert of the Bt11 event should be taken into consideration for the evaluation of the food safety of Bt11 (3) concerning the insert, no sound demonstration of the equivalence between the two types of maize is given at molecular/biochemical level and (4) potential differences might result in differences at compositional and toxicological level. The experts concluded that *"It remains impossible to state that the data of the studies done for Bt11 field maize are also valid for Bt11 sweet maize, given the absence of demonstration of molecular equivalence of the insert"*.

It was therefore decided that the notifier should prove the equivalence of the two maize lines by providing (1) the *in planta* genomic DNA sequence of the insert in Bt11 sweet maize, (2) an alignment of the DNA sequences of the field maize and the sweet maize, in correlation with the used transformation vector and (3) if sequence differences are observed between those two genomic DNA sequences, the impact of the observed differences on the nature and allergenicity of any of the recombinant proteins produced in the sweet maize product should be assessed. The request was sent to the notifier on 19 December 2003.

As a reply to this question, provided on 9 January 2004, the notifier stated that *"As there is no reason to suspect that varieties of sweet maize are more susceptible to genetic rearrangement than varieties of field maize, we believe that the sequencing of Bt11 field maize is applicable to Bt11 sweet maize. We have sequenced the genomic insert of Bt11, in some cases more than once and have used advanced breeding material"*.

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<sup>4</sup> No unintended ORFs were identified. However, the bio-informatic studies proving the absence of the ORFs are not included in the notification and should be provided.



## Conclusion

Taken into consideration that:

1. The molecular data of the genetically modified Bt11 field maize fulfils the current Belgian guidelines concerning the molecular requirements.
2. Based on sound scientific evidence, there is no reason to believe that there are differences at the molecular level in relation to the insert between Bt11 sweet maize and Bt11 field maize.
3. However, the notifier did not provide the information definitively demonstrating the homology of the Bt11 sweet maize and field maize inserts as requested by the Biosafety Advisory Council.

Consequently, The Biosafety Advisory Council postpones its positive advice until the requested information is provided.

## Other considerations

The Biosafety Advisory Council considers genomic sequence data as a corner-stone of the characterisation and the assessment of GMOs. As a consequence, the Biosafety Advisory Council considers that any modification of these sequences during traditional breeding should be considered as new scientific information which might influence the assessment of the GMO. The Biosafety Advisory Council will work towards further guidelines in this matter.

~~Prof. Dr. ir. Dirk REHEUL~~

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President of the Biosafety Advisory Council.

*Annex 1 : Expertise report. (ref: BAC\_2004\_FF\_115)*



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**Biosafety Advisory  
Council**



**Secretariat**

O./ref.: WIV-ISP/BAC/2004\_FF\_115<sup>1</sup>

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**Report of the Group of Experts mandated by the Biosafety Advisory Council  
(meeting of 17 December 2003 and meeting with the President on 17 February 2004)**

April 1st, 2004

**Evaluation of the application concerning the use of Bt11 sweet maize for food purposes  
as fresh vegetable or after processing by the applicant Novartis seeds, B.V.**

Scientific coordination by the Service of Biosafety and Biotechnology  
Secretariat of the Biosafety Advisory Council  
Scientific Institute of Public Health

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<sup>1</sup> Replaces document BAC\_2004\_FF\_113



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## 1. Context

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ON 11 FEBRUARY 1999, SYNGENTA, FORMERLY NOVARTIS, SUBMITTED A REQUEST UNDER REGULATION (EC) NO. 258/97 TO THE COMPETENT AUTHORITIES OF THE NETHERLANDS FOR PLACING SWEET CORN Bt11 ON THE MARKET AS A NOVEL FOOD OR AS A NOVEL FOOD INGREDIENT AND CURRENTLY THE APPLICATION IS IN THE PROCESS OF APPROVAL/DISAPPROVAL FOR AUTHORISATION.

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## 2. Introduction

In February 2003, analysis of the molecular data of the notification of the genetically modified maize Bt11 line, by the experts of the Biosafety Advisory Council *a.i.*<sup>2</sup> showed that the data on molecular characterisation of the Bt11 event do not fulfil the Belgian requirements<sup>3</sup>. The DNA sequence of the insert and the flanking regions was missing. In addition, no analysis of the flanking regions of the insert for the presence of chimaeric open reading frames was done.

Moreover, data provided by different scientific institutions suggested that there are still ambiguities concerning the molecular data of Bt11<sup>2</sup>. Namely, rearrangements in the insert and truncations of parts of the insert might have occurred.

On 17 November 2003 and 9 January 2004 the notifier supplied more information on the molecular data to the Belgian competent authorities. On request of the authorities, the novel food application in particular with regard to the information on the molecular data of the transgenic Bt11 maize event was evaluated by the experts of the Biosafety Advisory Council. The scientific evaluation of this additional information is presented in this report.

### 2. Evaluation of molecular data of the transgenic maize Bt11 event.

The information package of November 2003 contains the sequence of the insert and the flanking DNA of the Bt11 maize event. In addition, the sequence of the insert provided by the notifier was aligned with the one determined by TEPRAL. Within the insert, a few nucleotides were found to be different between the two sequences. The notifier states that the sequence of the insert in Bt11 is 100% identical to that of the plasmid used for transformation.

From the presented data it was concluded that the data are sufficient to lift the main ambiguities (rearrangements, truncations, insertions that have occurred), but further information should be given to lift the doubts concerning the sequencing differences.

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<sup>2</sup> The members of the Biosafety Advisory Council were officially nominated on 6 May, 2003. Before that date, the Service of Biosafety and Biotechnology (SBB) acted as the Biosafety Advisory Council *a.i.*.

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Therefore, the experts recommended (1) to check the sequence of the insert by (an) independent laborator(y)ies in order to lift the doubts concerning the correctness of the sequencing data, and (2) that a comparison of the sequencing data provided by the notifier should be made with the sequence of the vector used for obtaining the transformation event maize Bt11.

In its information package of 9 January 2004, the notifier states that the genomic insert of Bt11 has been sequenced more than once. Also, the alignment of the Bt11 plant sequence with the sequence of the vector used for transformation was included. The information provided by the notifier was found to be sufficient to lift the remaining doubts concerning the correct sequence of the insert.

In addition, as a request by the Belgian Competent Authorities during the consultation period (60 days period) foreseen under Directive 2001/18/EC, the flanking regions of the Bt11 insert were analysed for the presence of chimaeric open reading frames. The notifier states that no unintended ORFs were identified. However, the bio-informatic studies proving the absence of the ORFs were not included.

In conclusion, the molecular data of the transgenic Bt11 maize event fulfil the Belgian requirements concerning molecular data, but the bio-informatic analysis should be provided.

### **3. Evaluation of Bt11 sweet maize application with regard to the molecular data**

The evaluation of the information in the dossier Bt11 sweet maize revealed that (1) the application presents data generated with Bt11 field maize and Bt11 sweet maize to support the food application, (2) since field maize and sweet maize are considered as safe, only the biosafety linked to the insert of the Bt11 event should be taken into consideration for the evaluation of the food safety of Bt11 (3) concerning the insert of Bt11, no sound demonstration of equivalence between the two types of maize is given at molecular/biochemical level and (4) potential differences at molecular/biochemical level might result in differences at compositional and toxicological level. The experts concluded that it remains impossible to state that the data of the studies done for Bt11 field maize are also valid for Bt11 sweet maize, given the absence of demonstration of molecular equivalence of the insert.

It was therefore decided that the notifier should prove the equivalence of the two maize lines by providing (1) the *in planta* genomic DNA sequence of the insert in Bt11 sweet maize, (2) an alignment of the DNA sequences of the field maize and the sweet maize, in correlation with the used transformation vector and (3) if sequence differences are observed between those two genomic DNA sequences, the impact of the observed differences on the nature and allergenicity of any of the recombinant proteins produced in the sweet maize product should be assessed.

As a reply to this question (given on 9 January 2004), the notifier stated "*As there is no reason to suspect that varieties of sweet maize are more susceptible to genetic rearrangement than varieties of field maize, we believe that the sequencing of Bt11 field maize is applicable to Bt11 sweet maize*".



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#### 4. Conclusions

The notifier should provide the bio-informatic studies done on the flanking regions of the insert of the transgenic Bt11 event in order to completely fulfil the Belgian requirements on molecular data.

Since in the reply of the notifier, the genomic DNA sequence of the transformation event Bt11 sweet maize is not given, none of the demands by the Belgian experts as mentioned above (see point 3) are fulfilled.

One could wonder why the questions of the Council were understood by the notifier as related to variety- specific frequencies of gene rearrangement and did provoke such a reaction.

As long as the equivalence of the molecular data in the separate dossiers has not been demonstrated, it remains impossible to accept that the data of the studies done for transgenic Bt11 field maize are also valid for transgenic Bt11 sweet maize.

In conclusion, some experts share the opinion that the notifier should provide the above mentioned information in order to allow the determination of equivalence between the materials used in the different analyses.



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