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Molecular characterization of GM event Bt-10

Summary report on scientific data obtained at the JRC-GMO-CRL and an analysis of the data on Bt-10, obtained by Syngenta.

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Executive summary

In April 2005, the JRC Community Reference Laboratory on GMOs in Food and Feed (CRL) was requested to carry out an in-house validation of an event specific method for Bt-10 maize, following the Commission Decision of 18 April 2005 (2005/317/EC) on "Emergency measures regarding the non-authorised genetically modified organism Bt-10 in maize products".

Syngenta, the developer of Bt-10, provided to the CRL only a partial DNA sequence of the Bt-10 construct which did for instance not include the DNA sequences of the flanking regions. Also other important information, such as a demonstration of a physical link of the event specific amplicon (which constitutes the diagnostic signal for the Bt-10 detection method) with the rest of the construct was missing. In the absence of such data and the need of such information for assuring effective implementation of the emergency measures, the B&GMOs unit started a characterisation of the Bt-10 event.

During the course of its research, the CRL noticed that control samples provided by Syngenta did not consist of pure Bt-10, but that they were contaminated with event Bt-11. Further analysis of the Bt-10 event therefore became difficult and only limited techniques for analysis could be used. Nevertheless, data was obtained and revealed various problematic issues concerning the presumed structure and organisation of the Bt-10 construct:

- It seemed that there was more than one copy of the *bla* sequence, of the *cry* sequence and also of the *pat* sequence.
- The organisation of the different elements, either the intactness of the elements or the positioning versus each other, was different than what was initially expected.
- The Bt-10 PCR detection method yields a 117 bp amplicon, containing part of the *bla* sequence. This amplicon could not be directly linked to the rest of the Bt-10 construct.

This data showed that the organisation of the Bt-10 construct was more complex and different from the data initially provided by Syngenta. It became clear that the Bt-10 event does not consist of one straightforward integration and that the positioning of the event specific amplicon with respect to the rest of the construct was not clear. Subsequently, these findings put in doubt the robustness of the Bt-10 detection method. If the Bt-10 elements, including the region which would yield the Bt-10 specific PCR amplicon, were to be scattered over the genome, this could lead to potential false negative results in Bt-10 screenings. Consequently, in March 2006, the European Commission expressed doubts on the robustness of the Bt-10 method and asked Syngenta for clarification concerning the molecular structure of Bt-10 and liability of the Bt-10 method. The company provided new data (dossier March 15, 2006), confirming a complex structure for Bt-10.

During a first meeting to discuss the molecular data (May 8, 2006) Syngenta reported that the DNA sequence, and hence the map of the organisation of Bt-10 which was provided

in the initial dossiers (April and June, 2005) was incorrect. Given the complexity of the Bt-10 insert, a precise map was unlikely to be technically achievable. Nevertheless, data were shown which suggested that all Bt-10 elements were located on one single locus. The JRC requested more detailed investigation of the linkage of the Bt-10 specific amplicon with the rest of the construct. These data would be crucial to decide on the robustness of the Bt-10 detection method.

Syngenta provided more precise data at a meeting with the JRC of September 18, 2006, indicating that all Bt-10 elements are positioned on one locus (of 24 kb), including the Bt-10 specific amplicon. The same integration pattern was found for individual plants tested, showing the nature of the insert to be consistent.

As final conclusion, it can be stated that the analysis of the Bt-10 event (carried out by Syngenta and the CRL) has shown that the insert has a complex molecular structure, many times larger than originally postulated; the insertion has not been a straightforward integration and re-arrangements have occurred. Due to this complex integration (full and partial copies of *cry*, *pat* and *amp*; several repetitive elements), a precise map of the Bt-10 organisation cannot be made. Although the event specific amplicon is not immediately linked to a full *cry* and *pat* sequence, it seems to be part of one locus together with the other Bt-10 elements. Therefore, it is reasonable to conclude that the Bt-10 event specific method, generating a 117 bp amplicon, is indicative for the presence of the Bt-10 construct.

The present status of the knowledge of the organisation of the insertion(s), leads the JRC-GMO-CRL to conclude that the issue of possible false-negatives has been sufficiently dealt with within the technical limits of the current state-of-the-art methodology.