COMMENTS TO THE ASSESSMENT REPORT OF THE UK COMPETENT AUTHORITY: NOTIFICATION C/GB/02/M3/3

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General aspects:

- There is no possibility to verify the given information, for lack of appropriate bibliographical references

Technical comments:

Molecular characterisation

In the assessment report incorrect informations have been reported:

- **Transformation technique of MON810,** "The transformation event has been produced by particle bombardment of genotype Hi-II with a 5.5 kb *Nde*I fragment of the plasmid PV-ZMBK07". Instead, MON 810 has been produced by transformation with with a DNA solution containing two plasmids: PV-ZMBK07 and PV-ZMGT10[1],
- **Molecular characterisation of MON 810,** "Nucleotide sequence analysis has determined that the inserted DNA sequence is identical to that of the donor plasmid vector.". Instead, a truncation of the Cry1Ab ORF is confirmed by DNA sequencing[2].

In the assessment report important informations have not been reported:

- **NK603**, sequencing analysis performed by the notifier have shown the presence of unexpected sequences adjacent to the insert, a 217 bp fragment of the rice actine promoter and a 305 bp with homology to chloroplast DNA;[3]
- **MON810**, the flanking sequence at the 3' end of the insert shows homology with sequences of mitochondrial origin[4], moreover sequence data and PCR analysis, reported by third party suggest a deletion and/or rearrangement of genomic plant DNA at the insertion site[5].

[1] Opinion of the Scientific Committee on Plants Regarding the Genetically Modified, Insect Resistant Maize Lines Notified by the Monsanto Company (NOTIFICATION C/F/95/12/02)
[2] Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/DE/02/9) for the placing on the market of insect-protected genetically modified maize MON 863 and MON 863 x MON 810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto, The EFSA Journal (2004) 49, 1-25
[3] SNIF C/ES/01/01
[4] Assessment Report of C/DE/02/09
[5] Hernandez M, Pla M, Esteve T, Prat S, Puigdomenech P and Ferrando A. A specific real-time quantitative PCR detection system for event MON810 in maize YieldGard based on the 3'-transgene integration sequence. *Transgenic Research* 2003, 12, 179-89.

In conclusion, we consider that:

- data reported by the UK Competent Authority are incomplete and/or incorrect, consequently the conclusions of the assessement report should be incorrect;
- it is not clear if, correct molecular analysis have been performed, to exclude the presence of plasmid backbone in genomic DNA of plant;
- the presence of unexpected sequence adjacent to the insertion site has not been assessed;
- transformation of maize by particle bombardment could induce genotypic alteration in the plant genome, however this hypothesis has not been investigated, it should be necessary to apply profiling technologies (transcriptomics, proteomics, metabolomics) in order to check unintended effect do the genetic modification.

Assessment of use in animal feeds

- It is essential that the tested protein is equivalent to the novel protein expressed in the GM plant. If a protein, which was produced by micro-organisms, is used, the structural and functional equivalence of the microbial substitute to the novel plant protein has to be demonstrate (comparison of the molecular weight, the isoelectric point, post-translational modification, immunological reactivity…). In the case of maize NK603 EPSPS protein and maize MON810 CRY1Ab protein, some differences in the amino acid sequences have been observed. It is not clear if the differences reported have been assessed;[6]

- statistically significant differences in the composition between GM line and non-transgenic control hybrid have been reported[7]. Even if these modification fell within the range reported for the non-GM test hybrid they suggest us that potential unintended effect due to genetic modification should be occur. So we require further evaluation to determine any biological significance;

- it should be appropriate to perform a feeding study with ruminants, indeed broiler chicken have a different and not comparable digestive apparatus to these. More animals should be tested to have more statistically significative values;

- Bacillus thuringiensis cannot be taken as allergenicity-free. In 1999, infact, researches discovered that workers developed positive skin tests and elevated specific IgE and IgG antibody levels to B.thuringiensis kurstaki (Btk) spore extracts containing Cry1Aa and Cry1Ab delta endotoxin proteins after respiratory exposure to Btk crop spraying (Bernstein et al, 1999). Another evidence were found last year in a village on the south of Philippines. Some villagers living near fields planted with Dekalb 818 YG – a hybrid between MON810 and a locally adapted variety (dekalb 818) – become ill when the maize started to flower. Dr. Terje Traavik , director of the Norwegian Institute of Gene Ecology, found antibodies reacting against the Bt toxin Cry1A(b), which is producted by MON810, in the sera of 39 farmers who were affected.[8] An integrated approach should therefore be used in the assessment of possible allergenicity of newly expressed proteins as described in EC Guidance Document for the risk assessment of GMP and derived food and feed.

[6] Assessment Report point 5.1 pag.7
[7] Assessment Report point 5.2 pag.7
[8] Traavik T. A response to criticism about our work on GE biosafety.2004

Assessment of environmental risk

- An indirect environmental impact due to the importation of maize seeds should be the co-importation of harmful lepidoptera resistant to bt toxin (Tabashnik, 1994)[9]

Surveillance and monitoring plane

- We consider necessary a monitoring plan targeted to control the presence of harmful lepidoptera resistant to bt toxin.

[9] Tabashnik B.E., Annual Review of Entomology, 39, 1994