***"The EFSA GMO Panel concludes that maize MON 89034 x 1507 x MON 88017 x 59122 is unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses."*** *– European Food Safety Authority, EFSA Journal 2010; 8(9):1781*

**Comments from European Member States during a three-month consultation period:**

"A stacked organism has to be regarded as a new event, even if no new modifications are introduced. The gene-cassette combination is new and only minor conclusions could be drawn from the assessment of the parental lines, since unexpected effects (e.g. synergistic effects of the newly introduced proteins) cannot automatically be excluded." – Austria, Federal Ministry of Health, page 1.

"Serious problems are not really expected by the application of MON 89034 x 1507 x MON 88017 x 59122. The safety aspects of the multiple challenge, due to the combination of the newly inserted proteins, are rather weakly demonstrated." - Belgian Biosafety Advisory Council, page 34.

"It is highly desirable to refer to studies that have demonstrated that the combination of all these newly inserted proteins is not detrimental." – Belgian Biosafety Advisory Council, page 38.

"The applicant concludes that based on the submitted risk assessment the adverse effects on humans and animals are negligible, and thus case-specific monitoring is not appropriate. However, this conclusion needs to be justified by more data addressing the concerns with regard to the presented risk assessment. Specifically, the possibility of synergistic effects of the transgenic proteins present in GM maize MON 89034 x 1507 x MON 88017 x 59122 should be addressed in an improved way." – Austria, Federal Ministry of Health, page 31.

"The Federal Agency for Nature Conservation considers that further information is required before the risk assessment of [SmartStax] can be finalized." – Germany, Federal Agency for Nature Conservation, page 50.

"a) there is no safe use of the new recombinant protein expressed by an artificially arranged insert such as Cry1A.105.
b) concerning all Bt toxins, a history of safe use cannot be argued on the basis of the safety of Bt sprays applied in organic farming. The inserted genes are truncated and arranged with expression modulating DNA parts originating from different organisms and permanently expressed compared to a tight timely Bt spraying schedule (Lewis et al. 1997; Sexton et al. 2007)." – Austria, Federal Ministry of Health, page 22.

**Comments Specific to Allergenicity**

"1) Assessment of the allergenicity of the newly expressed proteins.
It must be emphasized that Cry1A.105 displays high amino acid sequence identity with Cry1Ac and that Cry1Ac has been proposed as an adjuvant for vaccines (Vasquez et al, 1999, Vasquez-Padron et al. 1999, Moreno-Fieros et al. 2003, Esquivel-Perez et al. 2005), which means that this protein is able to enhance the immune responses against antigens that are co-administered, which is not uncommon for a bacterial protein. Other proteins of the Cry family are also

suspected of showing adjuvant properties (Calderon et al. 2007). Therefore, doubt may arise about Cry2Ab2, Cry1F, Cry3Bb1 and Cry34/35 Ab1. The consequence of the presence of such immuno-stimulant in a plant destined to human consumption is not known. Particularly the adjuvant effect via intestinal route is poorly documented. The single concentration of Cry1A.105 in maize grains is compatible with the possibility of an adjuvant effect in the context of normal maize grain consumption (but the concentration after processing of the maize or after cooking is not known). If all Cry proteins also have such adjuvant capacity, the adjuvant effect may be multiplied in MON89034x1507xMON88017x59122 maize. It is not known whether the presence of these Cry proteins in maize may elicit sensitization against the other maize proteins upon ingestion (and which type of sensitization?). This point needs to be clarified. Therefore, it is relevant to at least study in mice the immune responses against maize proteins when the animals are fed MON89034x1507xMON88017x59122 maize." - Belgium, Belgian Biosafety Advisory Council, Page 40.

**Needed Studies/Recommendations:**

1. A stacked organism has to be regulated as a new GM event. (p.1) Experiments with SmartStax should be carried out, data from the parental lines can be informative but not sufficient for the risk assessment. (p.59) Experiments should account for the total high amount of Bt protein and for the possible mixture of the proteins. (p.59)
2. At least one subchronic (90-days) feeding study with rodents is needed to examine immune responses. (p.40)
3. Supplemental studies with ruminants and swine which differ with respect to their digestive systems and which will be substantially exposed to feed derived from SmartStax. (p.58)
4. Multigenerational studies focusing on reproduction should be considered. (p.40)
5. Assessment of the expression of the transgenic proteins needs to be studied over several growing seasons, at least 2 in similar or comparable locations, to establish adequate baseline exposure data. (p.4)
6. Field plot sizes need to be bigger as to not limit the significance of results pertaining to ecological characteristics. (p.6)
7. Data on ecological relevant parameters e.g. duration of pollen, viability, flowering time or susceptibility towards pest and disease (investigated under pest or disease pressure) need to be gathered. (p6)
8. The genetic stability of the GM corn needs to be assessed. For example, an adequate number of individual plants should be analyzed with methods that allow the assessment of the integrity of the transgenic insertions and the flanking sequences. (p.16)
9. The pelleting process, silage process and distillation process (DDGS) need to be described in detail to assess whether or not they are likely to change the characteristics of the GM product compared with its non-GM counterpart. (p.20) Data on degradation during processing is needed. (p.61)
10. Studies to determine that the combination of newly inserted proteins is not resulting in synergistic effects.
11. More studies to assess the effects on non-target organisms. (p.50)
12. Test the influence of the application of glyphosate and glufosinate on the expression of the proteins. (p.54)

13. Estimates for human exposure should be thorough and include basic ingredients.