GREENPEACE

Greenpeace preliminary comments on Monsanto Roundup Ready maize, event NK603
Notification number under Directive 2001/18/EC : C/ES/00/01

Greenpeace thinks that, in the state of current knowledge and considering the scientific uncertainties as well as the risks involved, no GMO should be released into the environment or placed on the market. However, in particular, NK603 should be rejected on the following basis:

1) **there is clear evidence of a transcribed open reading frame at the 3' end of the sequence, raising the possibility that some additional, unintended protein is produced or there is modification to other maize proteins.**
2) **there is an unknown sequence with chloroplast homology, the consequences of which are not clear.**
3) **there is an amino acid change in one of the CP4 EPSPS proteins. This has not undergone any food safety evaluation.**
4) **In addition, there is insufficient data to determine whether there have been deletions or rearrangements within the maize genome. Should further details reveal deletions or rearrangements, this would be grounds for rejection.**
5) **and the copy number has been brought into question by other EU authorities on hybrid applications involving NK603 hybrids. Should there be multiple copy number, the consequences of this should be investigated thoroughly.**

1) Open Reading Frame
Monsanto (2002) states "In addition to the two complete *cp4 epsps* cassettes, corn event NK603 contains a 217 bp portion of DNA containing part of the enhancer region of the rice actin promoter at the 3' end of the inserted DNA in the inverse direction of the *cp4 epsps* cassettes. RT-PCR analyses were conducted across the 3' junction between the NK603 insert and the adjacent corn genomic DNA sequences to assess transcriptional activity. The results from these analyses demonstrated that mRNA transcription was detected to initiate in either one of the two promoters of the NK603 insert and proceed through the NOS 3' polyadenylation sequence and continue into the corn genomic DNA flanking the 3' end of the insert. This result is not unexpected since the incomplete termination or use of alternative termination sites and resulting production of multiple transcripts has been reported for endogenous genes in plants."

Thus, it appears that transcription carries on through the end of the insert (including the stop codon), through the additional fragment of the rice actin promoter and into the maize genome. **This raises the possibility that either a) an unintended novel protein could be produced or b) a maize protein may become modified.** Any such proteins produced would not be detected unless detailed protoemic comparisons between NK603 and the non GE sister line are carried out. It is not clear from the documents whether even routine 2D SDS page was carried out. **In addition, any such proteins produced**

would not have undergone any toxicity testing and therefore their food safety would be completely unknown.

Furthermore, Monsanto (2002) state: "Given the structure of the *cp4 epsps* coding sequence, the surrounding genetic elements and the nature of the plant's protein-producing machinery, any transcripts longer than full-length would either produce a CP4 EPSPS protein longer than the full-length protein or the full-length CP4 EPSPS protein itself. No longer than full-length CP4 EPSPS protein was detected as assessed by western blot analysis. Only the full-length CP4 EPSPS protein was observed. Therefore, it was concluded that only the full-length EPSPS proteins are produced in corn event NK603."

This statement does not adequately reflect the complex post transcriptional and post translational modifications that occur in eukaryotes. Some proteins are formed in large molecular precursors to proteins which are then cut to yield the final protein or proteins (see, e.g. Lewin, 2000). These are then cut down to a final protein or proteins by specialised enzymes or proteases. This process could occur within the protein(s) formed by the open reading frame (or at least, no evidence has been presented to suggest this has not happened). **Therefore, it is entirely possible that proteins other than full length CP4 EPSPS are produced by the open reading frame but these have not been detected as they have not been looked for.**

2) Sequence with homology to chloroplast

There is brief mention (Monsanto 2003) of a 305 bp sequence with homology to chloroplast DNA. There is simply inadequate information to assess the implications of this sequence. Chloroplast of what? **This may be critical, because, as the UK ACRE note: "chloroplast DNA can recombine at a greater frequency than indicated by the applicants" (ACRE, 2002**)

3) Change in nucleotide sequence

Monsanto (2002) states: "Nucleotide sequence of the insert showed that the *cp4 epsps* coding region regulated by the rice actin promoter was as expected. However, the *cp4 epsps* coding region regulated by the E35S promoter contained two nucleotide changes, one of which results in a change of the amino acid leucine to proline at position 214 in the protein. The CP4 EPSPS protein containing this change is referred to as CP4 EPSPS L214P. The other nucleotide change did not result in an amino acid change."

This nucleotide change has been completely unassessed for any toxicity or allergenicity implications as it is asserted (Monsanto, 2002) "the two CP4 EPSPS proteins are indistinguishable in western blot analysis with the available polyclonal antibody, since the proteins are essentially identical." **However, toxicity and allergenicity may be affected by the small changes in the amino acid sequence. There has been no assessment of the food safety for these changes.**

4) Insufficient detail on deletions or rearrangements

Monsanto (2002) "The sequences flanking the insert were confirmed to be native to corn.". The flanking sequences may well belong to the maize genome, but are they

contiguous? Where is the proof that there have been no deletions? In the notification (Monsanto 2003), it simply says "not applicable" under this section, which is insufficient. Some scientific examination of the possibility of deletion must be produced. This is particularly important as the transformation method used, particle bombardment, is now well known to give rise to multiple copy number, insert fragments as well as deletions and rearrangements of the recipient genome (see, e.g. Kohli, et al., 1998; Pawlowski et al., 1998; Dai et al., 2001).

5) Copy number

In a similar application (NK603 x MON810), UK ACRE requested additional information that "included clarification of the copy number of the NK603 insert … since the Southern blot presented could be interpreted ambiguously." It is not possible to evaluate this on the basis of the information given but should be carefully evaluated by those with access to the full dossiers.

Finally, it is interesting to note that more detailed information is available on NK603 from the Monsanto website than through the SNIF publications, even though SNIF are designed to inform the public.

Greenpeace International
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