Greenpeace technical comments on: SNIF for the deliberate release and placing on the EU market of

insect resistant Bt11 maize, Syngenta Seeds, C/FR/96/05/10 France

http://gmoinfo.jrc.it/csnifs/C-FR-96-05-10.pdf and http://gmoinfo.jrc.it/csnifs/C-FR-96-05-10_RiskAssessment.pdf

Updated 24th October 2003 to include new molecular data.

Greenpeace is opposed to the deliberate release of GE crops regardless whether the release occurs within or outside the EU. This application is for the cultivation of GE *Bt* maize intended for human and animal consumption. There are important implications for both the environment and human health associated with this maize which should result in a rejection of this application.

This particular application should be rejected on the grounds that:

1) **There is mounting scientific evidence that the growing of Bt crops has deleterious effects on the environment, including non-target insects. Bt11 is known to exude the Bt toxin from plant roots, which accumulates in soils.**
2) **There are many irregularities regarding the molecular data, including rearrangements, truncations and unexpected GE fragments. It appears that Bt11 has been contaminated by Bt176, possibly by cross-pollination.**
3) **The compositional data are of poor quality, with significant differences noted. Analytical data from field maize are submitted for consideration of sweet maize. Food safety is unknown and important as the maize may be eaten unprocessed by humans.**
4) **Allergenicity/toxicological data are limited.**
5) **Coexistence with non-GE maize is highly difficult and no plans are proposed to control volunteers or monitor contamination.**

1) Effects of growing Bt crops on the environment – including long term effects

There are many environmental concerns over the growing of *Bt* crops including: effects on non target organisms (including long term exposure), persistence and accumulation of *Bt* toxins in the soil and insect resistance to *Bt*. See, e.g. Greenpeace (2002) "Environmental Dangers of Insect Resistant *Bt* Crops" for a review of the scientific literature.

Research has suggested that transgenic *Bt* plants could also be harmful to non-target organisms that feed on pests exposed to their toxins. *Bt* toxins from GE maize could kill non-target species and be passed higher up the food chain. **The SNIF states (pg. 16, Q26) that "a large number of field trials have been conducted since 1992". However, the appendices list only 4 EU countries. What non-target species were examined? Over what time period? This is no basis upon which to assess whether there are effects on non-target organisms.**

The *Bt* toxins is exuded by roots of Bt crops, and has been studied in Bt11 (Saxena et al., 2002). does not degrade quickly but persists in the soil adsorbed onto soils particles, whilst remaining physiologically active. It has been shown that the *Bt* toxin persists and remains active in soil for up to over 200 days (Koskella & Stotzky, 1997; Tapp & Stotzky, 1998; Stotzky, 2000 and Zwahlen et al., 2003a). The long term, cumulative effects of continued growth over several years of GE plants expressing toxins are not well known but recent studies (Zwahlen et al., 2003b) have shown that there may be detrimental effects to soil fauna (earthworms) of long term exposure to *Bt*.

The SNIF does not state what concentrations of *Bt* are present in the roots of Syngenta's Bt11, even though it is known that the *Bt* toxin is exuded from the roots. The SNIF does not include any monitoring of the soil for build up of the *Bt* toxin.

The build up of insect resistance will affect organic farmers who use *Bt* sprays. The SNIF states (pg. 13, Q21) that "Generally, higher levels were detected at the younger stages of tissue development. The level of Btk protein decreased as the plant reached full maturity and the tissues became senescent" and the EC Scientific Committee on Food (2002) states: "The Cry1A(b) protein levels in husks and kernels were found to vary, showing no consistent trend." **Such variability and decreases in *Bt* concentrations throughout the growing season will increase the rate of insect resistance build up** (see for example, Knight, J. (2003)).

2) Rearrangements, truncations and unexpected fragments

Molecular characterisation of Bt11 have shown several irregularities (De Schrijver & Moens, 2003). **There are rearrangements of the primary insert, and several parts of the plasmid have been truncated or unexpectedly inserted.** In particular, fragments of t35S, a "stop" codon are present in the primary insert. t35S is not the stop codon used in Bt11, but is the stop codon used in Bt176. **It is suggested that a fragment of Bt176 is present in Bt11 as the result of contamination, possibly by cross-pollination.**

The flanking regions show that the genetic insert has integrated on a 180 bp knob specific tandem repeat sequence (Rønning et al. 2003), which was not stated in the dossier. The implications of insertion of the genetic construct into this important region are unknown. Knob DNA is an important part of the maize chromosome and has been shown to have multiple functions and influence several genetic effects, such as flowering time (Ananiev et al., 1998). In addition, knob DNA sequences are complex and controlled by several elements including mobile elements such as retrotransposons. Therefore, disruption of a knob sequence may interfere with the function of either the knob sequence itself or retrotransposons. The mobility and functionality of retrotransposons are dependent on several factors, including environmental factors. Therefore, any adverse effects relating to the interruption of a knob sequence may only become apparent under certain environmental conditions, e.g. drought. **The interruption of a knob sequence is could give rise to unexpected effects, genetic instability in future generations, and possibly alter important plant functions such as flowering time** (Jank &Haslberger, 2000).

3) Compositional analysis

The previous application for Syngenta's Bt11 was criticised by EU member states for insufficient analytical data of the maize to establish substantial equivalence. The dossier was criticised for pooling data from different locations; significant differences were noted with regard to some nutrients; analysis of secondary metabolites were from one location only; different hybrids were used and the statistical analysis inadequate (Schenkelaars Biotechnology Consultancy, 2001).

It is not clear to what extent additional data has been provided in the amended dossier, but it is clearly not satisfactory. **The EC Scientific Committee on Food (2002) states "These measurements were executed with the use of various hybrids, different experimental designs and sometimes with different analytical methods…The Committee is of the opinion that despite the large number of studies, the company did not commission systematic information on the composition of the genetically modified or control plants."**

The EC Scientific Committee on Food (2002) is at odds with the scientific concerns on member states as it states in it's assessment of the Bt11: "the Committee agrees that the distinction between the results of the sweet maize and field maize is not relevant for the assessment as long as the appropriate corresponding non-modified maize is used as control." Whereas, the UK ACNFP (2001) states in its letter to the European Commission "As it is likely that some of the Bt11 sweet maize will be eaten unprocessed, it is essential that before approval is given studies relating to the expression of the introduced genes are addressed and that data is provided on the sweet maize rather than from its parental field maize." Furthermore, the EC Scientific Committee on Plants (2000) stated, in their assessment of Bt11 stated that a lower protein content of two transgenic hybrids grown in the northern US was found. "According to the applicant, this difference is related to the back-crossing for producing the hybrids." **Therefore, it is evident that creating hybrids from GE events can lead to compositional changes. It is essential that data on sweet maize, rather than field maize should be presented.**

Significant differences in some of the components of some studies, such as palmitic acid, stearic acid, cystine and arginine are simply regarded as "not relevant" by the EC Scientific Committee on Food (2002). However, the EC Scientific Committee on Plants (2000) does not explicitly state that substantial equivalence has been achieved. **These significant differences and others noted in the original application should be investigated fully as the maize may well be eaten unprocessed by humans**.

4) Allergenicity/toxicity data

The UK ACNFP (2001) noted "Digestibility and acute oral toxicity studies on the Cry 1A protein were carried out on protein expressed in *E. coli* rather than the protein expressed in the sweet maize. Other *in vivo* studies were based on studies in tomato. Although the recent FAO WHO report on safety of GM foods derived from plants recognises the merits of testing material derived from analogous systems it stresses the need to demonstrate such material is biochemically and functionally equivalent to that produced in the genetically modified food. The applicant should be asked to demonstrate such equivalence."

It is not clear whether this has been achieved as the EC Scientific Committee on Food (2002) states "The toxicological information included data from digestibility studies with simulated gastric fluid and single dose oral toxicity studies with mice on the PAT and Cry1A(b) proteins. **Such studies provide only limited evidence for safety**."

5) Coexistence with conventional maize is highly difficult

GE maize is described as presenting a "medium to high risk" for cross pollination with conventional or organic maize (Treu & Emberlin, 2000). Thus, there is a high probability that conventional and organic maize could become contaminated with Bt11. It is acknowledged in the SNIF that maize seeds may be disseminated by harvesting, transport and wind damage (pg. 8, Q11). Although GE maize is not expected to become a persist or invasive weed, should any volunteer GE maize plants inadvertently grow near a conventional maize crop, the resulting pollen could cross-pollinate with maize in fields, producing genetic contamination. Maize plants have been shown to survive over a growing season, even in a comparatively cold part of Europe, the UK (Crawley et al., 2001) and this is acknowledged in the SNIF (pg. 8, Q10). Survival might be expected to be higher in warmer parts of Europe. Maize volunteers have been noted occasionally from spilled seed in uncultivated fields and by roadsides in the year following maize production (Eastham & Sweet, 2002). Who will control the volunteers and how? The spraying of roadsides, which are valuable wildlife habitats is simply not acceptable. There do not appear to be any plans to monitor or control such volunteers in the SNIF, nor contingency plans for contamination of non-GE maize.

References

Ananiev, E.V., Philips, R.L. & Rines, H.W. (1998) Complex structure of knob DNA on maize chromosome *9*: retrotransposon invasion into heterochromatin. *Genetics*, **149**, 2025-2037.

Crawley, M.J., Brown, S.L., Hails, R.S., Kohn, D.D. & Rees, M. (2001) Transgenic crops in natural habitats. Nature, 409, 682-683.

De Schrijver, A. & Moens, W. (2003) Report on the mo lecular characterisation of the genetic map of event Bt11. Available at: http://biosafety.ihe.be/TP/MGC_reports/Report_Bt11.pdf

Eastham, K. & Sweet, J. (2002) Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. Expert's Corner Series, European Environment Agency, Copenhagen.

EC Scientific Committee on Food (2002) Opinion of the Scientific Committee on Food on a request to place genetically modified sweet maize line Bt11 on the market

SCF/CS/NF/DOS/14 ADD2 Final, 6 May 2002. http://www.europa.eu.int/comm/food/fs/sc/scf/out129_en.pdf

EC Scientific Committee on Plants (2000) Opinion of the Scientific Committee on Plants on the submission for placing on the market of genetically modified insect resistant and glufosinate ammonium tolerant (Bt-11) maize for cultivation. Notified by Novartis Seeds SA Company (notification C/F/96/05-10) (opinion adopted by the Scientific Committee on Plants on 30 November 2000). http://www.europa.eu.int/comm/food/fs/sc/scp/out86_gmo_en.html

Greenpeace (2002) "Environmental Dangers of Insect Resistant *Bt* Crops. December 2002 http://www.greenpeace.org/international_en//reports/more-reports?start=9&archived=&campaign_id=3942

Jank. B. & Haslberger, A. (2000) Recombinant DNA insertion into plant retrotransposons. *Trends in Biotechnology*, **18**, 326-327.

Knight, J. (2003) Agency "ignoring its advisers" over *Bt* maize. *Science*, **422**, 5.

Koskella, J. & Stotzky, G. 1997. Microbial utilization of free and clay-bound insecticidal toxins from *Bacillus thuringiensis* and their retention of insecticidal activity after incubation with microbes. *Applied and Environmental Microbiology* 63: 3561-3568

Saxena, D., Flores, S. & Stotzky, G. (2002) Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. *Soil Biology & Biochemistry*, **34**, 133-137.

Schenkelaars Biotechnology Consultancy, 2001 GM food crops and application of substantial equivalence in the European Union. Available at: http://www.sbcbiotech.nl/

Stotzky, G. (2000) Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. *Journal of Environmental Quality*, **29**, 691-705.

Rønning, S.B., Vaïtilingom, M. Berdal, K.G. & Holst-jensen, A. (2003) Event specific real-time quantitative PR for genetically modified Bt11 maize (*Zea mays*). *European Food Research & Technology*, **216**, 347-354.

Tapp, H. & Stotzky, G.. 1998. Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. *Soil Biology & Biochemistry* 30(4): 471-476.

Treu, R. & Emberlin, J. (2000). Pollen dispersal in the crops Maize *(Zea may*s), Oil seed rape *(Brassica napus* ssp *oleifer*a), Potatoes *(Solanum tuberosu*m), Sugar beet *(Beta vulgaris* ssp *vulgari*s) and wheat *(Triticum aestivu*m). A report for the Soil Association from the National Pollen Research Unit. Available at http://www.soilassociation.org

Zwahlen, C. Hilbeck, A. Gugerli, P. & Nentwig, W. (2003a) Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. *Molecular Ecology*, **12**, 765-775.

Zwahlen, C. Hilbeck, A., Howald, R. & Nentwig, W. (2003b) Effects of transgenic *Bt* corn litter on the earthworm *Lumbricus terrestris. Molecular Ecology*, **12**, 1077 –1086.

UK ACNFP (2001) Advisory Committee on Novel Foods and Processes (ACNFP) Annual Report 2000. Appendix 4, http://www.foodstandards.gov.uk/science/ouradvisors/novelfood/acnfpannual