Opinion of the Scientific Committee on Plants on the Invocation by Austria of Article 16 ('safeguard' clause) of Council Directive 90/220/EEC with respect to the placing on the market of the Monsanto genetically modified maize (MON810) expressing the Bt cryia(b) gene, notification C/F/95/12-02 (Opinion expressed by the Scientific Committee on Plants on 24 September 1999)

Background

The Scientific Committee on Plants was consulted by the Commission on the dossier for a genetically modified maize derived from MON810 transformed to express the Bt **cry**IA(b) gene for tolerance to insect damage, and published its opinion on 10 February 1998 (SCP 1998). A Commission Decision for the placing on the market of this maize (C/F/95/12-02) was adopted on 22 April 1998 and the French authorities issued the corresponding consent on 3 August 1998.

The Commission received notification from the Austrian authorities of their decision to invoke Article 16 of Directive 90/220/EEC on 2 June 1999. This informed the Commission that the marketing of genetically modified maize, MON810, was to be prohibited by an ordinance that would enter into force within the following two weeks. The Austrian Competent Authority took the decision to invoke Article 16 following a re-evaluation of this notification and specific data published in a recent scientific study, which addressed possible adverse effects of pollen from genetically modified Bt-maize on the monarch butterfly. The Scientific Committee on Plants has been asked to advise the Commission on the following aspects:

(a) Whether the information submitted by Austria constitutes relevant scientific information that was not taken into account by the SCP at the time its opinion was delivered?

(b) Whether the information constitutes relevant scientific information that invalidates the original risk assessment for this product?

(c) Whether this information constitutes relevant scientific information that invalidates the original risk assessments for the other Bt-products that have been approved or are pending approval following the SCP's appraisal?

(d) Would this information cause the Committee to consider that these Bt-products constitute a risk to human health and the environment, including non-target organisms such as butterflies?

Comment

Austria's reservations regarding the evaluation of the maize line MON810 are principally about the undesired effects of the Bt toxin on non-target organisms and the possible development of resistance in insects e.g. the European corn borer, which is the main target pest.

Bacillus thuringiensis is a very widely distributed bacterium in the soil and the phylloplane (e.g. Mizuki **et al.**, 1999) which produces crystals of protein within its cytoplasm which may have insecticidal toxicity. These crystalline proteins or ï • ¤-endotoxins are broken down by enzymes in the gut of some insects to liberate the active toxin which then destroys the gut wall leading to the death of the larval insect. Five classes of proteins are recognised (but revised nomenclature [see Crickmore **et al.** http://www.sussex.ac.uk/lifesci/btlab/index includes at least 22 different Cry types and CRY proteins) but the CRYIA proteins or protoxins are specifically toxic to lepidoptera and have been used safely in preparations as crop protection biopesticide sprays for some 40 years. When the gene for the CRYIA protein is incorporated into genetically modified maize or another crop, the protein can be expressed in the appropriate tissues of the plant and is therefore available selectively to the pest lepidopteran species consuming those tissues as it damages the plant. In addition, the most sensitive stage, the emerging larvae, will be targeted as they commence feeding on the Bt-modified plant.

Undesired effects on certain non-target arthropods

A number of tri-trophic laboratory studies have been carried out with non-target insects where genetically modified plants or artificial diet have been fed to herbivorous larvae subjected to predation or parasitism. Some studies have reported effects while others have reported no effects on predator or parasitoid. These results are difficult to interpret and extrapolate to field conditions. Scientific and technical issues in the laboratory that need further attention include the difficulty of reproducing realistic field exposure levels and routes and achieving experimental rigor to allow for the effects of reduced growth in affected herbivorous prey. Such interpretation must be viewed against the comparative risk assessment of alternative spray applications of insecticides.

Monarch butterfly study

A recent study by Losey **et al.** (1999) published in Nature reported that the larvae of the monarch butterfly, **Danaus plexippus,** when fed in laboratory designed and implemented experiments on the leaves of their natural host plant, milkweed **Asclepias curassavica,** artificially coated with pollen from Bt-maize, ate less, grew more slowly and showed higher mortality than similar larvae which fed on leaves free from pollen. The SCP considers that the reported studies are preliminary and raise a number of important questions, which have not been addressed. Comparison was made between leaves coated with GM pollen, those with pollen from an unrelated non-GM maize variety and leaves without any added pollen. However the non-GM pollen used as control did not come from an isogenic variety of maize and therefore did not test whether the pollen was apparently toxic in the absence of the gene. There was no measurement of the amount or biological activity of the pollen dusted onto the leaves. No information was provided on dose-response to help interpret the significance of the reported results and it is not clear whether the effects seen arose from direct toxicity or anti-feedant effects or both in this experiment where larvae had no choice of food. The Committee concluded that it is not possible to extrapolate the results of this initial laboratory study to the field situation and agree with the authors that further work is needed to investigate and verify such effects in the field.

Other lepidoptera

The Monarch butterfly is a North American species and has been only exceptionally recorded in Europe. However the Committee considered the wider implications of the results of this study for non-target lepidoptera in Europe. Whilst the CRYIA toxins are toxic to a large number of lepidopteran species, the sensitivity in laboratory studies varies considerably up to 10^4 fold according to species. The CRYIA protoxins produced by the Bt bacteria are polypeptides, which are proteolytically split in the insect's gut to release the smaller active toxin. Of the 4 modified maize lines that have had the **cry**IA(b) gene incorporated, MON809 and MON810 express the entire CRYIA protoxin while BT176 and BT11 express a truncated form of this protein, which may have a modified spectrum of activity compared with the native bacterial derivative. The Committee recognises that, in the same way as spray formulations of Bt containing the CRYIA toxin, genetically modified maize has the potential to be toxic to certain species of lepidoptera but this may vary with species. Indeed Peacock **et al.**(1998) concluded that it is difficult to generalise when predicting the susceptibility of native lepidopterans to Bt and the issue of susceptibility must be dealt with on a species-to-species basis.

Cultivated fields are not considered as important reproductive areas for lepidopterans other than species, chiefly some moths, which are economically important pests of agricultural crops. Lepidoptera egg sites and food plants are primarily to be found outside the cropped area, in field margins and more distantly in non-cropped habitats.

The food plants are considered unlikely to be exposed to significant quantities of pollen for reasons discussed below. Additionally larvae under field conditions are likely to be presented with some element of choice in the selection of food plant, which was not available in the design of the study of Losey **et al.** in which larvae were not given choice of food.

Maize pollen

Maize, a species largely pollinated by wind and gravity (i.e. is anemophilous), amongst the grass family has the largest pollen, which is round and relatively wet (50 to 60% water) and therefore tends not to stick to surfaces naturally. Pollen viability varies from a few hours or less at high temperature to a few days. Cool temperatures and high humidity are important factors in extending pollen life.

The risk of toxicity and exposure to non-target lepidopteran larvae in the field will depend on factors such as the level of expression of the protein in the released pollen, the quantities of pollen produced, its time and period of release, the dispersion and dilution of released pollen with distance, photodegradation of the Bt toxin, the washing effects of rain or dewfall and the larva's choice of food plant.

There are many reported studies of pollen dispersal, which have demonstrated that in high winds, some pollen may travel long distance. However, most of the released pollen is deposited close to the crop plant and there is a very steep deposition gradient away from source. In low to moderate winds it has been estimated that, compared with pollen concentrations at 1m from the source, approximately 2% is recorded at 60m, 1.1% at 200m and 0.75-0.5% at 500m (Emberlin **et al.**1999). At 10m from the field on average the number of pollen grains per unit area is ten times less than that observed 1m from the edge. Under European conditions maize produces pollen from mid-July to mid-August. Although any field of maize may release pollen over a period of up to 13 days, an individual plant will be active for perhaps half of this. Although many species of non-target lepidoptera may have larval stages of development during the months of July and August (Stradling 1999) when maize plants will produce their pollen, locally any potential exposure will be temporally limited in relation to the dynamics of the insect population. To summarise, the two relevant and prominent features of pollen release by maize are the time-restricted release and the rapid fall off in dispersion from the plant.

Bt products

There are three genetically modified maize lines, which have been approved to date:

- 1. Bt-maize tolerant to glufosinate ammonium (BT176) from Ciba-Geigy (C/F/94/11-03). The **cry**IA(b) Bt gene is expressed in pollen as well as all green parts of the plant and stems at levels 2-5 ppm fresh weight, but not in the silk or the seeds.
- 2. Bt-maize expressing the **cry**IA(b) Bt gene (MON810) from Monsanto (C/F/95/12-02). Toxin is expressed in vegetative tissues at levels of 4.5 - 9.2 ppm fresh weight, but only at 0.09 ppm fresh weight in pollen.
- 3. Bt-maize tolerant to glufosinate ammonium (BT 11) from Novartis (C/GB/96/M4/1) expressing the **cry**IA(b) gene in leaves, tassels, silk and seed but only at trace levels in pollen, <0.09 ppm (at the lower limit of detection).

Pending approval:

- 4. Bt-maize expressing the **cry**IA(b) gene (MON809) from Pioneer (C/F/95/12-01/B). The protoxin has not been detected in pollen.
- 5. Bt-cotton expressing the **cry**IA(c) gene (line 531) from Monsanto (C/ES/96/02).

Bt resistance

The SCP discussed with expert entomologists in southern Europe and published an opinion on 4 March 1999 advising the Commission on the necessary field monitoring and laboratory studies necessary to detect the development of any resistance to Bt in the field during the introduction of Bt crops (SCP 1999). This was aimed at the European corn borer **Ostrinia nubilalis**, the prime target pest but also included an action plan for the Mediterranean Corn Borer, **Sesamia nonagrioides.** The SCP considered and advised on the establishment of non-Bt refuges adjacent to modified crops but pointed out that, in view of the slow introduction into Europe, crops would be surrounded by natural refuges for some time to come. Monitoring also needs to cover those secondary pests, which may become more important economically through the local control of the primary pest species.

Conclusion

In the context of the accepted practices for the natural or commercial cultivation of maize crops and taking into account (1) the rapid decline in the deposition of maize pollen away from the crop, (2) the relatively short period of pollen release in relation to the timing of local butterfly reproduction and larval feeding, and (3) considering the reported results of the preliminary but inconclusive laboratory-based study, the Committee concludes that there is no reason to change its previous advice to the Commission on the risk assessments of the Bt crops which it has evaluated to date. As already publicly stated, the SCP considers that it would be sensible to conduct monitoring in post-release situations. Furthermore, it strongly endorses the practice of monitoring with appropriate and adequately targeted methodology, the large-scale introduction of such crops in order to detect any deleterious impact on non-target lepidoptera and other insect populations. The SCP wishes to be informed of the results of any such field monitoring studies and as relevant information continues to become available will further advise the Commission and draw its attention to any significant concerns that may arise.

Summary

The Scientific Committee concludes that the information submitted by Austria does not constitute new significant information that was not already considered in its original risk assessment and opinion on MON810 and that the previous risk assessment stands unchanged.

The SCP also concludes that this information does not invalidate its original risk assessments for the other Bt products.

References

Emberlin, J., B. Adams-Groom and J. Tidmarsh (1999). A report on the dispersal of maize pollen. National Pollen Research Unit for the Soil Association.

Losey, J. E., L. S. Rayor and M. E. Carter (1999). Transgenic pollen harms monarch larvae. **Nature** (399): 214

Mizuki, E., T. Ichimatsu, S-H. Hwang, Y. S. Park, H. Saitoh, K. Higuchi, and M. Ohba, (1999). Ubiquity of **Bacillus thuringiensis** on phylloplanes of arboreous and herbaceous plants in Japan. **Journal of Applied Microbiology 86**, 979-984.

Peacock, J. W., D. F. Schweitzer, J. L. Carter and N. R. Dubois (1998). Laboratory assessment of the effects of **Bacillus thuringiensis** on native lepidoptera. **Environmental Entomology 27**, 450-457.

SCP (1998). 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). 16 February 1998.

SCP (1999). Opinion of the SCP on Bt-resistance monitoring. 4 March 1999.

Stradling, D. J. (1999). Report on the potential for exposure of British lepidoptera to toxic GM maize pollen. June 1999 for Greenpeace.