Opinion on the invocation by Germany of Article 16 of Council 90/220/EEC regarding the genetically modified BT-MAIZE LINE CG 00256-176 notified by CIBA-GEIGY (now NOVARTIS), notification C/F/94/11-03 (SCP/GMO/276Final - 9 November 2000) (Opinion adopted by written procedure following the SCP meeting of 22 September 2000)

1. TITLE

Opinion of the Scientific Committee on Plants on the invocation by Germany of Article 16 ('safeguard' clause) of Council Directive 90/220/EEC regarding the genetically modified Bt-maize line CG 00256-176 notified by Ciba-Geigy (now Novartis), notification C/F/94/11-03.

(Opinion adopted by written procedure following the SCP meeting of 22 September 2000).

2. TERMS OF REFERENCE

The Commission has asked the Scientific Committee on Plants (SCP) to consider the following:

- 1) Does the information submitted by Germany constitute relevant scientific evidence, which would cause the Committee to consider that this product constitutes a risk to human health and the environment?
- 2) Does this information constitute relevant scientific information that invalidates the original risk assessment for the other Bt-products that have been approved or are pending appraisal by the SCP?

3. BACKGROUND

In 1996 the Commission consulted three of its Scientific Committees on the dossier for a genetically modified Bt-maize line CG 00256-176 (Event 176) and its progeny transformed to express the Bt *cry*1A(b) gene for tolerance to insect damage. The Scientific Committee for Pesticides (the fore-runner of the current Scientific Committee on Plants) published its favourable opinion on 9 December 1996 (SCP 1996); both the Scientific Committee for Food and the Scientific Committee for Animal Nutrition published favourable opinions on 13 December 1996 (SCF 1996, SCAN 1996). A Commission Decision (97/98/EC [1]) to place this maize and its progeny on the market and permitting unrestricted cultivation (C/F/94/11-03) was subsequently adopted on 23 January 1997. The French authorities issued the corresponding consent on 4 February 1997.

Following notification from the Austrian Authorities of their decision to invoke Article 16 of Directive 90/220/EEC, all three Scientific Committees published further opinions confirming their original risk assessments. The Scientific Committee for Pesticides published its opinion on 12 May 1997 (SCP 1997); the Scientific Committee for Food published its opinion on 21

March 1997 (SCF 1997) and the Scientific Committee for Animal Nutrition published its opinion on 10 April 1997 (SCAN 1997).

The Commission received notification from the German Competent Authority on 4 April 2000 and 28 April 2000 of its decision to invoke Article 16 of Directive 90/220/EEC on 31 March 2000. This informed the Commission that the placing on the market of the genetically modified maize line CG 00256-176 and its progeny is suspended in Germany unless cultivation is intended for research and testing purposes in one of the following areas: effects on non-target or target organisms, the development of resistance, counter measures to resistance development, horizontal or vertical gene transfer, ecological assessments or the enhancement of agronomic and plant protection knowledge for practical application. For this reason Germany limits the amount of traded seed to 12 tonnes/year. The German Competent Authority took the decision to invoke Article 16 because of the suspicion that the preconditions for the placing on the market are not met for uses other than those specified above and where there is no restriction on volume.

4. OPINION

***Question*:**

The Commission has asked the Scientific Committee on Plants (SCP) to consider the following:

- **1) Does the information submitted by Germany constitute relevant scientific evidence, which would cause the Committee to consider that this product constitutes a risk to human health and the environment?**
- **2) Does this information constitute relevant scientific information that invalidates the original risk assessment for the other Bt-products that have been approved or are pending appraisal by the SCP?**

***Opinion*:**

The Scientific Committee on Plants has examined the scientific information provided by the German Competent Authority and does not consider that this alters the original risk assessments carried out on the Ciba-Geigy (now Novartis) Bt-maize Line CG 00256-176. The Committee also considers that the information does not invalidate the original risk assessments made for the other Bt-products which have been approved or are pending approval after evaluation by the SCP.

Scientific background on which the opinion is based

The Ciba-Geigy (now Novartis) modified Bt-maize line, CG 00256-176, contains the Bt *cry*1A(b) gene which confers insecticidal properties, the *bar* gene conferring tolerance to the herbicide glufosinate ammonium, and the *bla* gene as an antibiotic marker used in selection. The German Competent Authorities have provided information in four main areas, which are considered below.

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 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 see revised nomenclature [2]) and include at least 22 different Cry types and CRY proteins but the CRY1A 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 CRY1A protein is incorporated into genetically modified maize or another crop, the protein, when expressed in the appropriate tissues of the plant, is available selectively to the pest lepidopteran species which consumes those tissues as it damages the plant. In addition the emerging larvae which is the most sensitive stage will be targeted as they commence feeding on the Bt-modified plant.

4.1 Undesired effects on non-target arthropods

A number of laboratory studies have been published which have investigated the effects of Bt-modified plants or Bt-toxins in artificial diet fed to the larvae of target pests or other model insect species. Some have reported effects from tritrophic studies of herbivorous larvae and their insect predators or parasitoids whilst others have not detected any significant differences from controls. The implications of such laboratory experiments are very difficult to interpret and extrapolate to the field situation where a wide range of other factors may come into play. For example, in the laboratory it is difficult to achieve realistic field exposures and to introduce sufficient experimental rigour to allow for the effects of reduced growth in herbivorous larvae used as prey for predatory species. The series of papers by Hillbeck *et al.* (1998a, 1998b and 1999) illustrate these difficulties. The wider field significance of individual studies is further complicated by the different expression levels and distribution of Bt-endotoxins in the tissues of modified crop plants.

In a recently published study, Losey *et al.* (1999) examined the mortality of monarch butterfly (*Danaus plexippus* L.) larvae fed Bt-pollen on their milkweed food plants in the laboratory. The Committee has already considered this study and concluded that it is not possible to extrapolate to the field from these preliminary results (SCP 1999b). Lack of realistic and quantified exposure levels limit the interpretation of the study. A further recent semi-field and laboratory study by Wraight *et al.* (2000) measured the mortality of the larvae of black swallowtail butterflies (*Papilio polyxenes*) on potted host plants placed in or near Bt-maize. This showed no relationship between mortality and proximity either to the field (Pioneer 34R07 containing MON810) or to pollen deposition on the host plants and the conclusion drawn was that Bt-pollen of the variety tested is unlikely to affect wild populations of black swallowtails. Most recently, Hansen and Obrycki (2000) found significant larval mortality of monarch larvae fed on host plants exposed to Bt-pollen concentrations representative of those in the field for Bt-176 and MON810. However analytical results of toxin levels in the Bt-pollen used in the experiment were variable and differed from the expected toxin levels published elsewhere (EPA 1999a, EPA 1999b).

The implications of such studies have to be considered against the level of expression of Bt-toxin in pollen of the different Bt-maizes, the local timing and duration of pollen release in relation to the life cycles and development of lepidopteran larvae and the rapid decline of pollen deposition with distance from the source crop (SCP 1999b). In particular the interpretation and prediction of effects in the field should be viewed against the comparative risk assessment of alternative crop protection practices and exposure to insecticide sprays. The SCP concludes that the studies cited in the German submission do not invalidate the original risk assessments.

4.2 Development of resistance to Bt

The SCP discussed with expert entomologists in southern Europe and published an opinion on 4 March 1999 advising the Commission on the 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 1999a). 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.

4.3 Toxin release to soil

The issue of possible toxin release to soil has been the subject of recent literature reports. In summary, there is no evidence that Bt-endotoxins partition into the soil in a significant way from Bt-crops, or that Bt-proteins are in a form that normally would not be degraded by soil microbial communities. Furthermore, it is important to note that soils are the natural habitat of all Bt-species and that protein turnover occurs routinely in soils as part of the cyclic transformation of organic matter. Therefore, the stability of Bt-endotoxins in soil would not be expected to show different patterns of degradation compared to other proteins or DNA deposited in soil and subjected to interaction or binding with soil constituents during the microbial degradation process. Consequently, the SCP does not consider that there is evidence to demonstrate that soil-bound Bt-toxins from Bt-crops will persist and have additive adverse effects on numerous non-target organisms.

4.4 Antibiotic resistance

4.4.1 *Background*

In its letter of 4 April 2000 the German Competent Authority cites the opinion of Eco-Institute e.V. Freiburg, according to which the possibility of the ampicillin resistance gene spreading from the Bt-maize line CG 00256-176 to bacteria thus increasing the resistance to ï • ¢-lactam antibiotics cannot be excluded. The letter, however, concedes that "on the bases of current knowledge, unacceptable adverse effects are not to be expected in respect of the specified cultivation purposes if the quantity sown is limited to 12 tonnes/year, particularly in view of the already widespread resistance in bacteria to ï • ¢-lactam antibiotics".

The report of the Eco-Institute does not actually show any new assessment of the magnitude of the actual risk of antibiotic resistance genes being transferred from GM plants to bacteria. The report concentrates on the evaluation of the clinical significance of the antibiotics used as marker genes in GMO construction. The chapter on antibiotics affected by ampicillin resistance (*bla*r) gene lists the present uses of different penicillins and their derivatives used in veterinary or human medicine, and concludes that they still have a major importance in medical practice.

Since no actually new data are presented in the material attached to the German invocation, the risk of the antibiotic resistance gene present in CG 00256-176 has to be assessed on the

basis of the material originally presented by the company (Notification C/F/94/11-03 and associated documents) against the present knowledge on the horizontal gene transfer.

4.4.2 *The nature of the blar gene present in CG 00256-176*

The ***blar*** gene present in CG 00256-176 is from a well-known *Escherichia coli* vector PUC 18, a derivative of pBR322, one of the classic vectors used in molecular biology. The construct present in the plant also contains the origin of replication (*ori*) of the plasmid. The ***blar*** gene, coding for TEM ï • ¢-lactamase, originates from transposon Tn *A* and is present also in several other species and genera of bacteria. It confers resistance to a variety of penicillins and cephalosporins.

4.4.3 *Considerations by the notifier on occurrence and consequences of the ampr (bla) gene transfer*

The company produced several documents in 1995/6 reporting both experimental and theoretical estimates of the possibility and consequences of the *bla*r gene being transformed into rumen or gut bacteria. Briefly, their arguments can be summarised as follows:

- Transformation is the only way by which this gene could get introduced from plant material into bacteria.
- Even if transformed the gene should either be integrated into the host chromosome or stay associated with the *ori* region in a re-circularised structure recognised by the DNA-replication machinery of the host in order to survive and get expressed.
- Physiological transformation is a rare event, and requires intact high molecular weight DNA.
- The DNA present in the plant is rapidly degraded and inactivated both in the silage making process and in the conditions prevailing in the gastrointestinal tract.
- DNA in CG 00256-176 leaf homogenates was degraded very rapidly when homogenates were incubated at 37 °C, and failed to transform either competent or non-competent *E. coli* cells to Ampr phenotype. The result was the same even when intact DNA was used. The frequency of a competent *E. coli* being transformed by plant DNA was calculated to be less than 5.6 x 10-9.
- Even in the very rare event of transformation (and subsequent expression of the gene) actually taking place there would be no consequences to human or veterinary medicine due to the high prevalence of ampicillin resistant strain already in the environment and among rumen and intestinal bacterial isolates.

4.4.4 *The validity of the company arguments against the background of the present knowledge*

The experiments reported by the company appear competently done. It might be argued that the experimental setting with a single bacterium, *E. coli*, as a recipient in the transformation experiments represents poorly the situation in the rumen and intestine with the multitude of other genera and species present. However, since the *bla*r gene and the *ori*-sequence were both from *E. coli*, the experiment can be considered as a worst case scenario.

However, since the transfer of phage DNA from feed to the somatic cells of mice has been shown to occur (Schubbert *et al*. 1997, 1998) the statements by the company, based on *in vitro* data, may be overconfident. The occurrence of horizontal gene transfer cannot be overruled as a possibility.

Given the present uncertainties about the frequency of DNA transfer in the intestinal tract between food components, gut micro-organisms and host itself, the eventual consequences of such an occurrence involving the *bla*r gene are relevant for the risk assessment. Here the original company statements of the widespread occurrence of ampicillin resistance appears valid. The data cited on the relatively common occurrence of ampicillin resistance among bacterial isolates from farm animals is extensive, and there is no indication of any change in the situation during the recent years (i.e. Adesiuyn *et al.* 1998, Al Ghamdi *et al*. 1999, Farrington *et al.* 1999, Seyfarth *et al.* 1997). The numbers of ampicillin resistant strains resulting from a gene transfer from a GM-plant to bacteria would be insignificant against the background of already existing resistant micro-organisms.

4.4.5 *Conclusion*

Although the frequency of horizontal gene transfer between the GM-maize and the ruminal or intestinal bacteria may have been underestimated, the significance of such an event in this particular case would be negligible given regard to the high background presence of the *bla*r gene in the environment. Consequently there is no need to reconsider the previous Committee opinion on CG-00256-176 in this respect.

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

- 1. Bt-maize tolerant to glufosinate ammonium (BT176) from Ciba-Geigy [notification C/F/94/11-03]. The cryIA(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. (Approved for placing on the market and cultivation [3])
- 2. Bt-maize expressing the cryIA(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. (Approved for placing on the market and cultivation [4]).
- 3. Bt-maize tolerant to glufosinate ammonium (BT-11) from Novartis [notification C/GB/96/M4/1] expressing the cryIA(b) gene in leaves, tassels, silk and seed but only at trace levels in pollen, <0.09 ppm (at the lower limit of detection). (Approved for placing on the market but not for cultivation [5]).
- Two others Bt-maize lines and one Bt-cotton line have been evaluated by the SCP and are pending approval:
- 4. Bt-maize expressing the cryIA(b) gene (MON809) from Pioneer (C/F/95/12-01/B). The protoxin has not been detected in pollen. (Opinion of the SCP adopted on 19 May 1998.)
- 5. Bt-cotton expressing the cryIA(c) gene (line 531) from Monsanto (C/ES/96/02). (Opinion of the SCP adopted on 28 July 1998.)
- 6. Conventionally derived crosses between approved genetically modified maize lines T25 and Bt-MON810 from Pioneer Hi-Bred International Inc. as represented by Pioneer Overseas Corporation (Notification C/NL/98/08). (Opinion of the SCP adopted on 14 July 2000.)

5. REFERENCES

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- 11. SCF 1997. Opinion on the additional information from the Austrian Authorities concerning the marketing of Ciba Geigy maize (Opinion expressed on 21 march 1997).
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- 16. SCP 1999a. Opinion of the Scientific Committee on Plants on Bt-resistance monitoring (Opinion expressed on 4 march 1999).
- 17. SCP 1999b. 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 *cry*1(b) gene, notification C/F/95/12-02 (Opinion expressed on Plants on 24 September 1999).

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6. DOCUMENTATION MADE AVAILABLE TO THE COMMITTEE

Invocation of article 16 by Germany of Council Directive 90/220/EEC regarding a genetically modified Bt-maize line CG 0256-176 notified by CIBA-GEIGY (now Novartis), notification C/F/94/11-03, [Doc. SCP/GMO/262] comprising the following papers:

- An English translation of the notification of the Robert Koch Institute to the European Commission (DG ENV.).
- "Transgenic pollen harms monarch larvae" - in Nature Vol. 399, 20 May 1999 p. 214.
- "Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae)", Hilbeck A., Moar W.J., Pustzai-Carey M., Filippini A. and Bigler F. - in Environmental Entomology, Vol. 27, 1255 - 1263, 1998a.
- "Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae)", Hilbeck A., Moar W.J., Pustzai-Carey M., Filippini A. and Bigler F. - in Entomological Society of America, p. 480 April 1998b.
- "Prey-mediated effects of Cry1Ab and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea*", Hilbeck A., Moar W.J., Pustzai-Carey M., Filippini A. and Bigler F. - in Entomologica Experimentis et Applicata, 91(2): 305 - 310 - 1999.
- "Insecticidal toxin in root exudates from Bt-corn", Saxena D., FLorest S. and Stotzky G., in Nature Vol. 402, 2 December 1999, p. 480.
- Report: "Short expertise: Therapeutical relevance of antibiotic in connection with the use of antibiotic resistance genes in transgenic plants" -Baier A., Tappeser B., Öko-Institut e.V. - Institute of applied ecology, December 1999.

7. ACKNOWEDGEMENTS

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GMO Working group: Prof. O'Gara (Chairman) and Committee members: Prof. Davies, Dr. Delcour-Firquet, Dr. Hans, Prof. Hardy, Prof. Karenlampi, Mr. Koepp, Dr Kuiper, Prof. Silva-Fernandes, Dr. Speijers and invited experts: Dr. Aumaitre, Dr. Chesson, Prof. Moseley, Prof. Puigdomenech Rossell, Prof. Vighi and Prof. von Wright.

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[1] OJ L 131, 1. 2.1997, p. 69.

[2] see Crickmore et al. http://www.sussex.ac.uk/profiles/9816/research

[3] Commission Decision 97/98/EC, OJ L 31, 1. 2.1997, p. 69.

[4] Commission Decision 98/294/EC, OJ L 131, 5. 5.1998, p. 32.

[5] Commission Decision 98/292/EC, OJ L 131, 5. 5. 1998, p. 28.