REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE SAFETY FOR ANIMALS OF CERTAIN GENETICALLY MODIFIED MAIZE LINES NOTIFIED BY CIBA-GEIGY IN ACCORDANCE WITH DIRECTIVE 90/220/EEC FOR FEEDINGSTUFF USE

(Opinion expressed: 13 December 1996)

TERMS OF REFERENCE (October 1996)

The Scientific Committee for Animal Nutrition (SCAN) is requested to confirm that there is no reason to believe that the genetic modification of the maize lines covered by the Proposal of the Commission COM(96) 206 final, will give rise to any adverse effects on animal health when used in animal feed.

BACKGROUND

1. Council Directive 90/220/EEC[1] establishes provisions to protect human health and the environment when placing on the market products containing, or consisting of, genetically modified organisms intended for subsequent deliberate release into the environment.

2. On 15 March 1995 the Commission received a notification by the company Ciba-Geigy concerning the placing on the market of genetically modified maize, forwarded by the French competent authorities. The competent authorities of seven Member States raised objections on various grounds. In accordance with the procedure laid down in Article 21 of Directive 90/220/EEC the Commission submitted to the Regulatory Committee established by Directive 90/220/EEC a Proposal for a Commission Decision by written procedure on 8 March 1996. This Proposal sought to grant consent for the placing on the market of the genetically modified lines and any other maize (progeny) derived from crosses of these lines with traditionally bred maize.

3. On 11 April 1996 the Regulatory Committee foreseen by Article 21 failed to deliver an opinion on the measures proposed by the Commission. The objections of the Member States that relate to animal health concern the safety of the prokaryotic *bla* (beta-lactamase) gene introduced in the plant genome under the regulation of a prokaryotic promoter.

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1 Of 23 April 1990 on the deliberate release into the environment of genetically modified organisms (O.J. No. L117, 8/5/90 p. 15) as modified by Commission Directive 94/15/EC of 15 April 1994 adapting to technical progress for the first time Council Directive 90/220/EEC on the deliberate release into the environment of genetically modified organisms (O.J. No. L103, 22/4/94 p. 20)

4. Following the failure of the Regulatory Committee to deliver an opinion, the Commission forwarded to the Council a Proposal (COM/96/206 final) concerning the measures to be taken. The measures included in the Proposal for a Council Decision were identical to the ones presented to the Committee.

5. At the Environment Council of 25 June 1996 the Presidency concluded that the Council had drawn no conclusions and that this would allow both the French Government and the Commission to reflect on the issue.

6. Since Austria had provided further information concerning the safety of this genetically modified maize, on 24 July 1996 the Commission decided to ask three existing Scientific Committees to confirm the scientific basis of its Proposal. These Committees are the Scientific Committee for Animal Nutrition, the Scientific Committee for Food and the Scientific Committee for Pesticides.

OPINION OF THE COMMITTEE

Following Article 7 of Decision 76/791/EEC[2], the SCAN decided to form a working group from among its members with the mandate to report to the Committee on its work on Question 88 by the Commission. This Working group reported to the 104th Plenary of the SCAN -6 November 1996- that in view of the new documents[3], it could not make any recommendation and that to advance in the examination of this matter it was considered necessary to clarify the possibility of transfer of the *bla* (â-lactamase) gene from plant to bacteria; the data concerning the replication rate of the gene and the scope of the risk beyond the use of ampicillin. The Working Group suggested to invite highly specialised experts to have a consultation in the points referred to above and following Article 8 of the decision creating SCAN, the Commission invited the experts to participate at a Joint meeting with the Scientific Committee for Food (SCF). The consultation was held on 6 December 1996.

Based on the deliberation of the Working group and in the results of the Consultation, the opinion of the Committee is the following:

1. Concerning the plant expression of the Bt toxin and glufosinate inactivating enzyme, the SCAN is aware of the fact that these issues have been evaluated by the SCF. The SCAN can concur with the

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2 Commission Decision 76/791/EEC of 24 September 1976 establishing a Scientific Committee for Animal Nutrition (O.J. NO. L279, 9/10/76 p. 35.)

3 CS/NF/MAIZE/14: Document from the Scientific Committee for Food on additional information from MAFF (UK) concerning the risk of transfer of the intact bla gene. Later distributed as CS/NF/MAIZE/17: Potential for transfer of ampicillin resistance gene from GMO maize: Some information/comments considered by the Advisory Committee for novel food and processes, MAFF (UK) during its earlier deliberations.

conclusion that these properties are considered as harmless, since the toxin has a long history of use against insect larvae and has also being shown not to be allergenic or harmful when ingested by higher animals. Moreover, Bt protein and glufosinate inactivating enzyme are just two proteins among many other proteins in maize and will be denatured and hydrolysed in the gut.

2. The maize also contains a marker gene that expresses resistance to ß-lactam antibiotics. This leads to the following question:

Is it plausible that the marker-gene in the maize could be transferred to bacteria that are naturally present in the gastrointestinal tract of animals, making these bacteria resistant to ß-lactam antibiotics ?

The possibility of this transfer and its potential clinical significance were the focus of a joint meeting with the Working group of the SCAN and the SCF to which the Commission invited experts having particular knowledge in this matter.

Specific questions were identified by the two Working groups (SCF and SCAN), and as a result of this meeting, the potential for increased ß-lactam antibiotics resistance from GM maize was evaluated as follows:

2.1 Classification of the pUC plasmids

It is evident that the pUC plasmids do not contain mobilizing genes, nor a basis for mobility and are therefore unable to mobilize themselves. pUC plasmids are considered safe cloning vectors and are classified in Class 1 according to Council Directive 90/219/EEC[4].

2.2 Characteristics of the *bla*-gene construct

The copy of the *bla*-gene construct (with origin of replication -ori-) in the maize has the predicted base-pair sequence.

2.3 Presence of the pUC DNA in maize at cropping, processing and digestion.

The bla-gene construct is present throughout the whole genetically modified maize plant. The plant DNA, including the bla-gene construct (the size of which is approximately 1600 base-pairs) is after

4 Of 23 April 1990 on the contained use of genetically modified micro-organisms (O. J. No. L117, 8/5/90, p.1.) as last amended by Directive 94/51/EC (O.J. No. L297, 18/11/94, p.29). In Class I, the recipient or parental micro-organism is unlikely to cause disease to humans, animals or plants; the nature of the vector and the insert is such that they do not endow the genetically modified micro-organism with a phenotype likely to cause disease to humans, animals or plants, or likely to cause adverse effects in the environment; the genetically modified micro-organism is unlikely to cause disease to humans,animals or plants and is unlikely to have adverse effects on the environment.

disruption of the plant material degraded due to plant nuclease activity.

Low pH conditions (e.g. silage) result in rapid DNA degradation while encapsulation or adsorption of DNA to particulate material may protect it.

Fragments of less than 500 base-pairs (a majority of less than 200 base-pairs) are released after disruption. Larger fragments can be identified but are rare and often artefacts.

DNA which is released in the intestinal tract after eating intact grain is subject to digestion by extracellular nucleases of feed, digestive tract and microbial origin.

Under these conditions, the chance for an intact *bla*-gene construct to stay intact and available for transfer is consequently extremely low, if not zero.

2.4 Transfer of the *bla*-gene construct from maize into micro-organisms

Natural transformation is the only mechanism by which micro-organisms could acquire the *bla*-gene. Since the pUC plasmid has a very narrow host-range, because of the origin of replication, transformation could only be possible within the Enterobacteriaceae-Pseudomonas group.

Transformation requires competence of potential host bacteria which is a highly complex process markedly affected by growth conditions, age of cells and environmental conditions. According to the experts heard at the meeting of 6 December[5], there is no scientific evidence that ruminal or intestinal bacteria achieve naturally a competent state.

Another important component in the uptake process is the presence of multimeric forms of homologous DNA sequences at the same binding site on the cell surface. Therefore, in order to have bacterial uptake, multiple copies of the *bla* gene construct would have to emanate from the plant genome and aggregate at the binding site. These stringent requirements and the overwhelming amount of competitive DNA fragments make a natural transformation unlikely. Even under optimal experimental *in vitro* conditions, a successful transformation has not been achieved. Experts agreed that horizontal gene transfer from plant to prokaryotic organisms can be excluded on present scientific evidence.

Special attention was given to the aspect that the level of ß-lactamase produced and excreted by a bacterial cell containing the TEM gene

5 See reference 20.

with the pUC ori is considerably higher than that produced by a bacterial cell containing the TEM gene with another ori.

The experts were in agreement that the strains with the high copy number plasmid have growth disadvantages in comparison to the natural strains with a low copy number plasmid. High level expression could result in the bacterium being less competitive and unable to attain high numbers. In fact, an organism expending energy by expressing a *bla*-gene at a high level would be at a competitive disadvantage. This is probably the reason why the copy number of the Col E1 ori found in nature generates a maximum of 18 copies per cell.

2.5 Impact of the *bla*-gene encoded ß-lactamase resistance on therapy with antibiotics.

The pUC ß-lactamase is not capable of inactivating the isooxazolyl penicillins, cephalosporins of the newer generations and newer penicillins (e.g. ureidopenicillins) used in human medicine.

TEM1 ß-lactamase expressed from the bla-gene has a very narrow spectrum of resistance compared to variants now dominating *E. coli* strains in animals and humans. Genetic subsets of TEM1 exert a different resistance pattern.

TEM1 gene is common in material from animals or humans as well as in the contaminated environment. So nearly everybody harbours TEM1-gene containing bacteria at some period of life, through contact with the environment, e.g. with feed and food.

Not only is the TEM1 gene widely distributed in natural strains, but also laboratory strains with the TEM1 gene are particularly widely used without special precautions.

The clinical significance of the possible transfer of the TEM1-gene encoded
ß-lactam antibiotic resistance in animals is virtually zero.

2.6 Conclusion

Based upon the scientific knowledge available today and the following step-wise consideration:

a) that the probability of the transfer of a functional *bla*-gene construct from genetically modified maize into bacteria is virtually zero, and

b) that if the virtually impossible event occurred, it would have no clinical significance,

there is no evidence of a risk of causing ß-lactam antibiotic resistance in the bacteria of the animal digestive tract from the use of the genetically modified maize.

3. Overall assessment

The Commission requested the SCAN *"to confirm that there is no reason to believe that the genetic modification of the maize lines covered by the Proposal of the Commission COM(96) 206 final, will give rise to any adverse effects on animal health when used in animal feed"*. The SCAN's opinion is that there is no evidence indicating that the use in animal feeding of the genetically modified maize will give rise to any adverse effect on animal health.

REFERENCES

1. Proposal for a Council Decision concerning the placing on the market of genetically modified maize (*Zea mays L.*) with the combined modification for insecticidal properties conferred by the Bt-endotoxin gene and increased tolerance to the herbicide glufosinate ammonium pursuant to Council Directive 90/220/EEC (COM/96/206 final)

2. Full dossier submitted by Ciba-Geigy (Ref: CS/NF/MAIZE/1), in two volumes (Volume 1 consisting of Parts A, B1-9 and C1-11; Volume 2 consisting of Parts C12-18, D and E1-15).
N.B.: Of this full dossier, parts A to D being the same as those submitted to the French Competent Authorities in November 1994; part E being new and updated information submitted by the Company.

3. Summary of the full dossier submitted by Ciba-Geigy at the end of August 1996. (Ref. 29.VIII.1996 01; CS/NF/MAIZE/2).

4. Mailing between Member States, the Commission and Ciba-Geigy, consisting of comments, objections and responses. (Ref. : 15.III.1995 01; CS/NF/MAIZE/3).

5. Report of the vote of the Regulatory Committee, which includes statements from some Member States (Ref. 07/05/96/XI/007902; CS/NF/MAIZE/4).

6. Cover letter from the Austrian Authorities providing additional information to the Commission (Ref. 29.VIII.1996 01) and the following annexes :

   - Item 5 on cover letter : "Antibiotic Resistance Transfer in the Mammalian Intestinal Tract : Implications for Human Health, Food Safety and Biotechnology" (Cover page of book).

- Item 6 on cover letter : Article from TIBTECH "Accidental Release of Antibiotic-resistant genes".

- Item 7 on cover letter Letter regarding transfer of antibiotic resistance and an unofficial translation thereof.

Further references produced during the evaluation

7 SCAN/96/104: SCF guidelines for the assessment of novel foods

8 SCAN/96/115: U.S. Environment Protection Agency Pesticide Fact Sheet on Bacillus Thuringensis toxin sent by the Austrian Permanent Representation.

9 SCAN/96/119: Letter by two Austrian Ministers on Genetically Modified Maize

10 SCAN/96/121: Document on Genetically Modified Maize, including
1) Letter from O'Connor and Company on Bt maize, the US EC trade issues (20 September 1996)
2) Foundation for Nutritional Advancement and Tufts University: Antibiotic resistance via the food Chain. Case study: Ampicillin (Talloires, France. september 23-24, 1996.).

11 SCAN/96/125. Report by the ACNFP (UK) on antibiotic resistance markers in genetically modified plants for human food.

12. SCAN/96/135. Scientific Committee for Food: Additional information from MAFF, UK concerning the risk of transfer of the intact *bla* gene.

13. SCAN/96/140. DNA size in processed corn as estimated by PCR.

14. SCAN-info-37. Information received from FEFAC on Genetically Modified Maize (In German).

15. SCAN/96/150. Letter by the UK delegation on Maize Gluten Feed.

16. CS/NF/MAIZE/17. Potential for transfer of ampicillin resistance from GMO Maize.

17. CS/NF/MAIZE/18. The Scientific basis for the classification of the pUC-Plasmids according to Directive 90/219/EEC.

18 CS/NF/MAIZE/19. Information by Ciba-Geigy Limited relevant to the discussion on the ampicillin resistance marker gene.

19. Stellungnahme der Zentrale Kommission für Biologische Sicherheit (ZKBS) zur Mobilisierbarkeit des Plasmids pBR322 un dessen Derivativen

20. Transcript and report of an expert hearing on questions identified by the Scientific Committee for Food, and Scientific Committee for Animal Nutrition (SCAN) on the evaluation of the potential for increased ampicillin resistance from GMO Maize (CG-00526-176). Brussels 6 December 1996.