

# **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)**

## **1. TITLE**

**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)

Application for consent to place on the market and for cultivation, genetically-modified maize with Btk resistance to Lepidoptera and herbicide tolerance to glufosinate ammonium (*Bt-11*).

## **2. TERMS OF REFERENCE**

The Scientific Committee on Plants is asked to consider if the cultivation of this line of genetically modified maize within the European Community, and every variety derived by sexual reproduction with this line, is likely to cause any adverse effects on human health and the environment.

## **3. BACKGROUND**

Directive 90/220/EEC <sup>1</sup> requires an assessment to be carried out before a product containing or consisting of genetically modified organisms (GMOs) can be placed on the market. The aim of the assessment is to evaluate any risks to human health and the environment connected with the release of the GMOs.

Following the entry into force of the Regulation on Novel Foods and Novel Food Ingredients (EC No. 258/97) on 15 May 1997 <sup>2</sup>, in order for this maize seed and its derived products to be placed on the market for food purposes, the requirements of the Regulation will have to be satisfied. Such a regulation does not exist for novel feeds and novel feed ingredients.

The SCP published its opinion on this product on 10 February 1998 (under notification C/GB/96/M4/1) for the purpose of grain import and use only. The Commission published a favourable decision on the product on May 5<sup>th</sup> 1998 <sup>3</sup>.

The Animal and Plant Health Inspection Service (APHIS) of the USDA published positive conclusions in the Federal Register <sup>4</sup> by declaring that Bt-11 and any progeny derived from

hybrid crosses with other non-transformed corn varieties will be just as safe to grow as traditionally bred corn lines that are not regulated under 7CFR part 340.

## **4. OPINION**

### *Question*

**The Scientific Committee on Plants is asked to consider if the cultivation of this line of genetically modified maize within the European Community, and every variety derived by sexual reproduction with this line, is likely to cause any adverse effects on human health and the environment.**

### *Opinion of the Committee*

**The Committee is of the opinion that there is no evidence to indicate that the placing on the market for cultivation purposes of maize line *Bt-11* and varieties derived from this line by conventional crossing with maize lines other than genetically modified ones, is likely to cause adverse effects on human health and the environment.**

### *Scientific background on which the opinion is based:*

#### **4.1 Proposed uses**

The application is for the cultivation and marketing of genetically modified maize seed for all types of use (processing, food and feed production) like any other variety of conventional maize. Also covered are progenies developed by sexual reproduction with maize derived from the *Bt-11* transformation event. With respect to *Bt-11* maize and derived lines used as animal feed, both the whole crop (ensiled and fresh) and grains may be fed to livestock. Silage is made from fine - chopped whole plants, which are fermented anaerobically. Industrial products of maize processing, such as gluten feed, gluten meal, germ meal, and steep liquor, are also used as animal feed. Target species include cattle, pigs, poultry, sheep and goats, fish and companion animals.

#### **4.2 Description of the product**

The pollen of maize plants (*Zea mays* L.) derived from transformation event *Bt-11* was used to pollinate the female flowers of an inbred corn line. Descendants of the initial crossings have been successively back-crossed to evaluate different maize lines carrying the *Bt-11* event. Hybrid lines were produced. Maize grain which is the subject of this application for consent are produced from these hybrid lines and are therefore descended from the initial *Bt-11* transformation event.

#### **4.3. Molecular/Genetic Aspects**

##### **4.3.1. Transformation technique**

The genetic construct was introduced into protoplasts without a DNA carrier. Plants were then regenerated.

##### **4.3.2. Vector Construct**

The *Bt-11* transformation event has been obtained using plasmid pZO1502 containing the following components

- - a truncated synthetic *cry IA(b)* gene encoding *Btk* endotoxin. It also contains a synthetic *pat* gene (to allow transformant selection on glufosinate ammonium). 35S CaMV<sup>5</sup> is the promoter, *nos* 3' termination sequences are included and introns IVS 2 or IVS 6 are incorporated to enhance expression.
- - the plasmid pZO1502 contains the *ampR* gene used as selectable marker when the plasmid was generated in *E. coli*.
- - DNA from the well characterised plasmid pUC18 including portions of the *lac Z* and *lac i* genes and a segment of 1079 bp containing the bacterial origin of replication, *ori*.
- - Small pieces of DNA containing useful restriction endonuclease sites, inserted and used to combine the various components above.

#### 4.3.3. Transgenic construct in the genetically modified organism

The plasmid vector pZO1502 DNA was treated with the restriction endonuclease *NotI* in order to remove the *ampR* gene from the larger DNA fragment which contained the *Btk* gene fusion and *pat* gene fusion. This mixture of DNA fragments was then used to transform maize tissue.

The larger fragment contains the following:

- 1) the *pat* gene fusion (35S promoter - IVS 2 intron - PAT protein coding region - *nos* ' termination sequence), which allows production in plants of the PAT enzyme for resistance to the herbicide, glufosinate.
- 2) the *Btk* gene fusion (35S promoter - IVS 6 intron - *Btk* HD-1 protein coding region - *nos* 3' termination sequence), which allows production in plants of the *Btk* protein to protect the plant from damage by larvae of European Corn Borer.
- 3) DNA, totalling about 1,400 bp, and including a bacterial origin (*ori*) of replication, from the well characterised plasmid, pUC18.
- 4) small pieces of synthetic DNA, totalling about 120 bp and containing useful restriction endonuclease sites; inserted and used to combine the various components above.

The smaller fragment from the *NotI* digestion of pZO1502 contains the *ampR* gene. Southern blot and PCR<sup>6</sup> analyses have shown that lines derived from the initial *Bt-11* event do not carry the *ampR* gene. Thus, an antibiotic resistance gene is not present in the *Bt-11* event, nor in the maize grain or grain products produced from them. The *Btk* gene fusion and *pat* gene fusion are stably integrated as a single copy at a single locus in the long arm of chromosome 8.

## 4.4. Safety aspects

### 4.4.1. Potential for gene transfers

*Antibiotic (ampicillin) resistance gene - ampR* gene was used in the construction of the vector. Before the final transformation event it was, however, removed from the plasmid by cutting with a restriction endonuclease. Consequently, the resulting GM-plant does not contain the *ampR* gene.

*pat gene* - The gene is under the control of a plant promoter which is not functional in bacteria. Consequently, in the unlikely event of transformation, its expression would not occur. Even if, due to genetic recombination, the gene would be expressed in intestinal microorganisms or in human or animal cells, the probability of which is remote, no negative effects are expected because the only known substrate of phosphinothricin acetyltransferase (PAT) is the herbicide glufosinate ammonium.

#### 4.4.2. Safety of the gene product/metabolites (food and feed)

Grain produced from *Bt-11* maize hybrids contains Btk protein within the range of 4 to 5 mg/g fresh weight. The levels are approximately eight fold higher in leaves. PAT protein is present at around 50 ng/g fresh weight in leaves but is below detection levels in kernels. The analysis of Btk protein has been repeated on pollen and indicates lower levels than previously reported in the frame of the notification: 90 ng/g *versus* 125 ng/g

The notifier provided information on several toxicity studies performed in mammals and birds. Reference is made to three mice studies, in which mice were fed CRY1A(b) protein, or its truncated form, at up to 4000 mg / kg bodyweight without toxic effects. The notifier also cited evidence (Noteborn *et al.* 1994, ACS Symposium series 605: 134-147) from studies in which bacterial recombinant CRY1A(b) protein was fed to mice, rats, and rabbits without adverse effects.

Results of a bird feeding study are presented, in which bobwhite quail was fed CRY1A(b) enriched maize leaf protein. Quails (10 birds / group) received a single oral dose of 2000 mg maize leaf protein / kg bodyweight containing 700 µg/g CRY1A(b), corresponding to 1.4 mg CRY1A(b) / kg bodyweight. No effects were observed on mortality, feed consumption, body weight, and gross necropsy after 14 days following the single dosage.

Laying hens were also fed a diet for 14 days incorporating 64% corn meal from a *Bt-11* maize. The diet with transgenic corn contained 240 - 263 ng/g CRY1A(b) and 59 - 74 ng/g PAT. The control diet was slightly contaminated with transgenic kernels, accounting for 18 ng/g CRY1A(b). No significant differences were observed for feed intake, bodyweight, egg production, and egg weight. Egg whites and -yolks and tissue samples (breast, thigh, liver) have been analysed for CRY1A(b) and PAT at the end of the experimental period. Neither protein was found in these tissues above the detection limits of 6 ng/g for CRY1A(b) and 30 ng/g for PAT.

Four separate studies were made with cattle. The first, a short-term experiment with dairy cattle was designed to study any carry over of the CRY1A(b) and *pat* proteins into milk. Neither proteins could be detected in the milk of any animals fed whole-crop *Bt-11* maize. The second longer-term trial with dairy cattle compared performance of animals fed *Bt-11* maize or its conventional counterpart. There was no effect of the Bt trait on dry matter intake, milk production, milk composition or a number of rumen parameters relating to feed utilisation.

Beef steers allowed to graze residues of Bt and non-Bt maize showed no preference and when restricted to Bt or to non-Bt maize, no difference in daily weight gain was observed. However, this data is of limited value since the experimental design did not allow for differences in intake. A second trial involved 128 steer calves fed one of four silage-based diets using late and early maturing hybrids of Bt-maize and non-Bt maize. Although

differences due to variety were apparent, again no production difference attributed to the Bt trait was evident.

The weight of evidence provided by the company and available elsewhere leads the Committee to conclude that there is no significant risk to humans or livestock following ingestion of the introduced gene products. PAT and CRY1A(b) proteins are labile in *in vitro* assays with gastric juice of farm animals. Widespread use of natural Btk insecticides has not produced evidence of allergenic responses. Similarly no allergenic effect is predicted by comparing the new proteins (CRY1A(b) and PAT) with the structure of known allergenic proteins. However, the Committee is of the opinion that the often applied *in vitro* methodology to study the degradability of the Btk toxin (and phosphinotricin acetyl transferase) can be improved. In particular the use of the isolated protein in toxicity studies does not adequately model degradation of the same protein when fed as an integral component of the diet.

#### *Residue assessment:*

The principal residue identified in transgenic maize plants after post-emergence use of glufosinate ammonium was N-acetyl-glufosinate with lesser quantities of glufosinate and 3-[hydroxy(methyl)phosphinoyl]propionic acid (MPP) which is also found in non-transgenic plants. In maize grain, which exhibits much lower residues than the other plant parts, the principal residue identified was MPP with lesser amounts of N-acetyl-glufosinate. About 80 field trials are known to have been conducted with difference application rates in Europe and in the harvested grain the residue of each metabolite was below 0.05 mg/kg. In green maize, forage and fodder, higher residue can occur.

The glufosinate-derived residues do not concentrate in any maize processed fraction, which are relevant food or feed items. These include flour, starch, grits and oil. Residues are not detectable in crude and refined oil.

In ruminant and poultry feeding studies no detectable residues were found in meat, milk or eggs at the dose calculated to represent the highest residues in livestock feed under Good Agricultural Practices and taking into account the potential use of glufosinate herbicide in several tolerant crops.

It can be concluded, on the basis of the available data, that residues of glufosinate ammonium and its metabolites, N-acetyl-glufosinate and 3-[hydroxy(methyl)phosphinoyl]propionic acid (MPP) expressed as glufosinate free acid equivalents, will be below 0.1 mg/kg maize grain. In food of animal origin from livestock animal fed with feedstuffs after application of glufosinate herbicide in tolerant maize no residues above the limit of determination are to be expected.

There is no toxicological concern taking into account the ADI <sup>7</sup> of 0-0.02 mg/kg bw <sup>8</sup> for glufosinate-ammonium and N-acetyl-glufosinate and 3-[hydroxy(methyl)phosphinoyl]propionic acid, alone or in combination recommended by the WHO 1999 <sup>9</sup>.

#### **4.4.3. Substantial equivalence**

Maize kernels from glasshouse plants grown in Europe have been analysed for their composition. Moisture, nitrogen, ash, starch, cellulose, xanthophyll, and fatty acid- and amino acid compositions were determined. Kernels from four transgenic *Bt-11* maize hybrid lines and their non-transgenic counterparts grown in six US field locations in 1995 were analysed for protein, oil, starch, and fibre content. In addition, the fatty acid- and amino acid composition of kernels from two of these transgenic hybrid lines and their controls have been determined. Another two transgenic hybrid lines and controls from three US locations (1995) were analysed for copper, magnesium, manganese, zinc, folic acid, niacin, and vitamins B<sub>1</sub> and B<sub>2</sub>.

No significant differences for proximate compositions between transgenic plants and controls were found, except for the lower protein content of two transgenic hybrids grown in the northern US. According to the applicant, this difference is related to the back-crossing for producing the hybrids. In addition, minor differences are noted for palmitic and stearic acids, cystine and arginine contents.

Data have been supplied on three transgenic hybrid lines and their non-transgenic counterparts, which were grown at two locations in France in 1998. Their kernels have been analysed for proximate composition (carbohydrate, protein, fat, and fibre), fatty acid- and amino acid composition, and anti-nutritional factors (trypsin inhibitor and phytic acid). No significant differences were observed between transgenic and non-transgenic plants.

In a 1998 study in the US maize lines derived from the original *Bt-11* transformation event were analysed for nitrogen, carbohydrate, and lignin in experimental silage. Analysis indicated substantial equivalence of the GM lines with the non-GM controls with respect to silage composition. The silage was used in beef and dairy feeding trials.

#### **4.5 Environmental aspects**

##### **4.5.1. *Potential for gene transfer/gene escape.***

The risk of genetic escape from modified crop plants will be limited by poor dispersal and the absence of sexually-compatible plants either of the same or different species. *Zea mays* is not an invasive crop but is a weak competitor with limited powers of seed dispersal. Since pollen production and viability are unchanged by genetic modification in this wind-pollinated crop, dispersal and outcrossing frequency should be no different from other maize varieties. There are no plant species closely-related to maize in the wild in Europe and therefore the risk of genetic transfer to other species appears remote.

##### **4.5.2 . *Treatment of volunteers***

The risk of volunteer maize plants surviving is considered to be remote. In growing areas that are free from winter frost, which will kill any residual plants, any volunteers may be controlled by agronomic practices including cultivation and the use of a herbicide other than glufosinate.

##### **4.5.3 *Safety for non-target organisms***

The target pest is the European corn borer *Ostrinia nubilalis*, a pyralid moth, which feeds and develops within the maize stem. The *cryIa(b)* crystal proteins are specifically toxic to

*Lepidopteran* larvae on ingestion and appear non-toxic to other species of insects when consumed directly. Feeding studies conducted under laboratory conditions have produced variable results on the development of some predatory insect species (tritrophic studies) which have fed on prey that have fed on *Btk* material. Some have had no effect (Lozzia *et al.* 1998) while others have suggested an impact (Hillbeck 1998a, 1998b). The difficulty of achieving adequate nutritional status of the prey in laboratory studies questions the relevance to the real field situation. There are few published monitoring studies to date but Lozzia (1999) found no detectable differences in entomofauna between Italian fields of GM maize and adjacent fields of isogenic conventional maize.

The Btk-endotoxin is (and has been for some 20 years) applied widely as an agricultural pesticide against *Lepidopteran* larvae, often on a broad scale e.g. on maize and in forestry, in many EU member states, without detected changes in field populations of non-target insects. The CRY1A(b) protein in modified plants is identical to the same protein in microbial formulations used safely as crop-protection sprays. Direct feeding studies with pollen from GM maize have shown no effects on honeybee development, lady beetles, insidious flowerbug and green lacewing. The significance of recent preliminary feeding studies with monarch butterfly larvae and GM pollen was reviewed by the SCP in its opinion of 24 September 1999 <sup>10</sup>, which concluded that the results were not relevant to the field situation.

The SCP advises that insect populations in representative GM and similar non-GM maize crops should be monitored to provide reassurance that there are no effects on predators and parasitoids at an extended field scale. The SCP wishes to see the results.

Under the same growing conditions compositional data for grain and forage show that modified and unmodified plants are equivalent and no risk is identified to non-target herbivores including vertebrates. Direct feeding studies have shown no toxicity to birds (bobwhite quail and hens). Risks to soil organisms and soil function through degradation of modified plant material and contamination of ground water are considered to be extremely low. Laboratory feeding studies have not shown toxicity to earthworms and at field concentrations to *Collembola*.

#### **4.5.4 Resistance and tolerance issues**

The development of resistance in injurious target pests will be delayed by the rigorous adoption of a comprehensive resistance management strategy. To be effective this should require the active involvement of the notifying company to monitor for control failure, to provide technical support and to educate growers to implement the strategy.

The speed with which resistance to Btk toxin will develop in the target pest will depend on the rigour and efficiency of any insect resistance management strategy. Such a programme designed to delay resistance development requires adequate:

- 1) Knowledge of pest biology and ecology,
- 2) Gene deployment strategy (full-season, constitutive, optimal dose Btk expression to control insects heterozygous for resistance alleles),
- 3) Refuges to support the development of Btk toxin-susceptible insects,
- 4) Monitoring and reporting of incidents of pesticide resistance development,
- 5) Employment of integrated pest management practices that encourage ecosystem diversity and provide multiple tactics for insect control,

- 6) Communication and education plan,
- 7) Development and deployment of products with alternative modes of action.

Although it is not possible to determine optimal dose until resistant insects exist in the field, high protein levels appear present in all important plant tissues early in the season and should provide season-long control. The success of the resistance management strategy will depend on the ability of any monitoring programme to detect resistance as soon as possible and the extent and quality of advice given to farmers. The SCP published an opinion on 4 March 1999 on resistance monitoring <sup>11</sup> as developed by the Expert Group on Monitoring for Insect Resistance to Bt-toxins. Such monitoring should be carried out in Bt-maize and should provide an adequate framework to delay the onset of resistance in the target pest.

The SCP should be kept informed annually of the results of the proposed surveillance of resistance in the European corn borer in Member States. Separately the SCP welcomes the initiative to monitor all lines of Btk maize to be placed on the market for the development of insect resistance and wishes to be kept informed of progress.

In the absence of wild related plant species for potential genetic interchange, herbicide tolerance will not be a problem. The SCP understands that the notifier is currently not intending to market *Bt-11* hybrids as herbicide-tolerant hybrids.

#### 4.6 Conclusion (overall assessment)

The Commission requested the Scientific Committee on Plants to consider whether the placing on the market for cultivation purposes of genetically modified maize with Btk resistance to Lepidoptera and herbicide tolerance to glufosinate ammonium (*Bt-11*) and every variety derived by sexual reproduction with line *Bt-11*, is likely to cause any adverse effect on human health and the environment.

The Committee is of the opinion that there is no evidence to indicate that the placing on the market for cultivation purposes of maize line *Bt-11* and varieties derived from this line by conventional crosses between *Bt-11* line and maize lines other than genetically modified ones, is likely to cause adverse effects on human health and the environment.

The notifier has submitted a package of relevant information on grain and silage nutritional value and has carried out relevant feeding trials with target animals. Data from these studies provide no indication of risk associated with the cultivation of these GM maize lines.

The SCP should be kept informed of the results of monitoring and research studies in Member States with particular regard to the development of insect resistance.

## 5. REFERENCES

- 1. Noteborn et al. 1994, ACS Symposium series 605: 134-147.
- 2. FAO Plant production and protection paper 153, 1999 - IPCS, WHO/PCS/00.2.
- 3. [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-cryIIa gene, notification C/F/95/12-02 (Opinion expressed by the Scientific Committee on Plants on 24 September 1999).



- 4. [Opinion of the Scientific Committee on Plants on Bt-Resistance monitoring](#) (Opinion expressed on 4 March 1999)
- 5. Lozzia, G.C., Furlanis, C., Manachini, B. and Rigamonti, I.E. (1998). Effects of Bt corn on *Rhopalosiphum padi* L. (Rhynchotha Aphididae) and its predator *Chrysoperla carnea* Stephen (Neuroptera Chrysopidae). Bull. Zool. Agr. Bachic. Ser II. 30 (2): 153 - 164.
- 6. Lozzia G.C. (1999). Biodiversity and structure of ground beetle assemblages (Coleoptera Carabidae) in Bt corn and its effects on non-target insects. Bull. Zool. Agr. Bachic. Ser.II, 31 (1): 37 - 58.
- 7. Hilbeck A., Moar W.J., Pustzai-Carey M., Filippini A. and Bigler F. 1998a "Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae)", - in Environmental Entomology, Vol. 27, 1255 - 1263.
- 9. Hilbeck A., Moar W.J., Pustzai-Carey M., Filippini A. and Bigler F. 1998b "Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae)", - in Entomological Society of America, p. 480 April 1998.

## 6. DOCUMENTATION MADE AVAILABLE TO THE COMMITTEE

- 1. A dossier comprising:
  - A statement from the French competent authorities;
  - A report of evaluation prepared by the French competent authorities;
  - The complete dossier supporting the application of Novartis Seeds concerning the placing on the market of genetically modified maize (line Bt-11) for cultivation in the EU.
- 2. "Potential activity of Bt-maize against Monarch butterflies and other Lepidopterians in the EU: an assessment by Novartis Seeds (second part)" submitted by Novartis Seeds (Doc. SCP/GMO/169).
- 3. Response to questions (SCP/GMO/242-Rev1) to Novartis Seeds (Doc. SCP/GMO/249).
- 4. "Additional analyses on the composition of Bt-11 maize hybrids (freshly cut whole plants)" submitted by Novartis Seeds (Doc. SCP/GMO/254).
- 5. "Evaluation of transgenic event 176-Derived and Bt-11-derived "Bt" corn (maize) in the diet of lactating dairy cows", Final report, M.A. Faust, Iowa State University - submitted by Novartis Seeds (Doc. SCP/GMO/270 - study included also in the dossier referred to in reference 1).
- 6. Excerpt from Selected Proceedings from the Agricultural Biotechnology in the Global Marketplace Symposium, Baltimore, Maryland, July 2000 (Doc. SCP/GMO/279).
- 7. Preliminary report: research report to Novartis Seeds: Utilisation of Bt-corn residues in grazing beef steers and Bt-corn silage for growing beef cattle and lactating cows, Todd Milton and Rick Grant. (Doc. SCP/GMO/284) submitted by Novartis Seeds, 13 October 2000.
- 8. Preliminary Appendix 1 to the report "Utilization of Bt-Corn Residues in Grazing Beef Steers and Bt-Corn Silage for Growing Beef Cattle and Lactating Dairy Cows", Report no. NSB-012-00. (Doc. SCP/GMO/285) submitted by Novartis Seeds, 13 October 2000.
- 9. Preliminary report: Absence of transgenes and transgene products in tissues of animals fed diets containing transgenic plants or grain, L. Artim-Moore, M. Mullins,

M. Faust, D. Vlachos, L. Privalle. (Doc. SCP/GMO/286) submitted by Novartis Seeds,  
13 October 2000.

## **7. ACKNOWLEDGEMENTS**

The Committee wishes to acknowledge the contributions of the working group that prepared the initial draft opinion:

*GMO WG*: F. O'Gara (Chair) and Committee Members: H. Davies, M-P. Delcour-Firquet, R. Hans, A. Hardy, S. Kärenlampi, H. Kuiper, H. Koepp, A. Silva Fernandes, G. Speijers, and invited experts: L.-A. Aumaitre, A. Chesson, B. Moseley, M. Vighi and A. von Wright.

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<sup>1</sup> OJ N° L117 of 08.05.1990 p. 15.

<sup>2</sup> OJ N° L43 of 14.02.1997 p. 1.

<sup>3</sup> Decision 98/292/EC of 22 April 1998 - OJ L131/28.

<sup>4</sup> Vol. 61 N° 19 on 29 January 1996 (pp 2789-2790).

<sup>5</sup> Cauliflower Mosaic Virus.

<sup>6</sup> Polymerase chain reaction.

<sup>7</sup> Acceptable daily intake.

<sup>8</sup> Body weight.

<sup>9</sup> See ref. 2.