Opinion of the scientific committee on plants regarding conventionally derived crosses between approved genetically modified maize lines T25 and MON810 submitted by Pionner Hi-Bred International INC. as represented by Pioneer Overseas Corporation - Notification C/NL/98/08. (Opinion expressed by the Scientific Committee on Plants on 6 June 2000) (SCP/GMO/195-Final)

1. TERMS OF REFERENCE

The Scientific Committee on Plants (SCP) is asked to consider whether there is any reason to believe that the placing on the market of the T25xMON810 maize for handling and use as any other maize, excluding cultivation, is likely to cause any adverse effects on human health and the environment within the scope of Directive 90/220/EEC [1].

2. BACKGROUND

The original notification for T25 maize was submitted by AgrEvo Company under notification number C/F/95/12/07. The original notification for MON810 maize was submitted by Monsanto Company under notification number C/F/95/12/02. The SCP produced opinions on each notification that were published on 10 February 1998 [2]. The maize events T25 and MON810 have obtained consent to be placed on the market in the EU in accordance with Directive 90/220/EEC (Decisions 98/293/EC [3] and 98/294/EC [4]) . The consents covered crosses of either T25 or MON810 with traditionally bred (non-transgenic) maize lines. Therefore in this case, an additional risk assessment in accordance with Directive 90/220/EEC is required before a product derived from crosses between genetically modified organisms (GMOs), which have previously obtained consent, 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 new GMOs. For genetically modified plants, the assessment must be based on information outlined in Annex IIB to Directive 90/220/EEC and take account of the proposed uses of this product.

Following the entry into force of the Regulation on novel foods and novel food ingredients (EC No. 258/97 [5]) on 15 May 1997, 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 on novel feeds and novel feed ingredients.

3. DESCRIPTION OF THE PRODUCT

The product consists of maize T25xMON810 derived from conventional crosses. One inbred parent is derived from the progeny of line T25 (with increased tolerance to glufosinate ammonium-based herbicides due to the introduction of the **pat** gene from **Streptomyces viridochromogenes**). The other inbred parent is derived from the progeny of line MON810 (resistant to certain insect pests due to the introduction of the **cry1A(b)** gene of **Bacillus**

thuringiensis subsp. **kurstaki**). Detailed descriptions of the genetic elements present in lines T25 and MON810 are given in previous SCP opinions.

The notification also includes the progeny of crosses between T25xMON810 maize and any traditionally bred maize. All such crosses are referred as T25xMON810.

4. PROPOSED USES

The notification covers import of the genetically modified maize grain of T25xMON810 maize but excludes cultivation in the EU. Import is for use as any other maize including processing and feed uses.

5. OPINIONS OF THE COMMITTEE

5.1. Molecular/Genetic aspects

Since the product is the result of the crossing of two previously evaluated maize transformation events (T25 and MON810) the SCP has evaluated whether:

1) enough experimental evidence exists in the notification to confirm that the product is derived from a cross between T25 and MON810.

2) there are changes in the expression patterns of the introduced genes when compared with the original transformation events.

Concerning (1) the evidence presented is based on Southern blot analyses designed to compare the patterns of integration of the **pat** and **cry1A(b)** genes in T25xMON810 with those obtained for T25 (**pat** gene) or MON810 (**cry1A(b)** gene). Analyses used a number of different restriction enzymes and showed no differences in the integration patterns, indicating that the T25xMON810 maize is the result of crossing events between T25 and MON810.

Concerning (2), detailed quantitative ELISA of **pat** and **cry1A(b)** protein levels have been carried out with the T25xMON 810 hybrid and a comparison made with values from parental T25 or MON810 lines. The analysis revealed no significant differences in levels of protein expression.

5.2. Safety aspects

The issues related to gene transfer have been already addressed in the evaluation of the parental lines. No additional gene is present in the hybrid.

5.2.1 Safety of gene products/metabolites (feed aspects)

The safety issues regarding the presence of **pat** and **cry1a(b)** genes and their products in maize lines T25 and MON810 intended for use in feed have already been addressed in the relevant and separate notifications for T25 and MON810. Confirmation that concentrations of the gene products **pat** and **cry1a(b)** are similar in the hybrid T25xMON810 and its parent lines has been obtained. In 1997, average levels of **pat** in grains were 5.7 and 5.8 mg/kg for T25xMON810 and T25, respectively. **Cry1a(b)** was present at concentrations of 46 and 37 mg/kg in grains of T25xMON810 and MON810, respectively. As the presence of both genes

within the hybrid does not change the levels of their expression and as there is no reason to expect an interaction between the introduced genes, no additional health risks are introduced.

5.2.2 Residue assessment for parental T25 line

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-methylphosphinico-propionic acid (MPP), which is also found in non-transgenic plants. In maize grain, which exhibits much lower levels of residues than the other plant parts, the principal residue identified was MPP with lesser amounts of N-acetyl-glufosinate. In maize grain, approximately 5% of 300 samples analysed in US trials exhibited residues ³ 0.05 mg / kg. About 80 field trials were conducted in Europe with different application rates and in the harvested grain the residue of each metabolite was < 0.05 mg / kg. The glufosinate-derived residues do not concentrate in any fractions of processed maize, which are relevant to food or feed items such as flour, starch, grits or oil. Residues are not detectable in crude and refined oil.

It can be concluded on the basis of the available data that residues of glufosinate ammonium and its metabolites, N-acetyl-glufosinate and 3-methylphosphinico-propionic acid, expressed as glufosinate free acid equivalents, will be below 0.2 mg / kg in imported field maize grain. No residues above the limit of detection are to be expected in food of animal origin derived from livestock fed with GM maize treated with glufosinate herbicide.

5.2.3 Substantial equivalence

Compositional analyses have been carried out with maize obtained from field trials conducted in 1997 and 1998. Data from a 1997 US field trial, in which gross protein, fibre, fat, moisture, ash, fatty acid and amino acid composition were analysed in grains of lines T25, MON810, T25xMON810 [and a control line (hybrid 3394)], show that apart from small differences in fatty acid composition, no significant differences occur between the different lines. Similar analyses were carried out on grains from field trials conducted at four locations in Italy in 1998, with additional measurements of minerals (Ca, K, Mg, Mn, Na, P), vitamins (folic acid, thiamine, riboflavin, vitamin E), and anti-nutrients (phytate, trypsin inhibitor). In these trials, the hybrid T25xMON810 line (hybrid X1106RT) was compared with an equivalent control (cv. Cecilia). Statistical analysis of this data revealed some significant but small differences between T25xMON810 and Cecilia in grain protein, ash, stearic acid, linolenic acid, arginine, and riboflavin content. However, the levels of these components were within the ranges published for maize. In addition, the data presented for grains in the 1998 trial are almost entirely comparable to the values provided for the 1997 US field trial. In conclusion, the compositional data obtained from the 1997 and 1998 field trials demonstrate the substantial equivalency of hybrid T25xMON810 to its parent lines T25 and MON810 and to the non-transgenic conventional maize except for the transferred traits.

5.3. Environmental aspects

5.3.1 Potential for gene escape

Since this notification does not include the cultivation of maize in the EC, the only potential release is by spillage of grain during transport or processing. Maize grain is relatively heavy and in the unlikely event of spilled grain becoming established in non-cropped habitats, **Zea**

mays is not invasive but is a weak competitor with limited powers of seed dispersal. There are no closely related wild plants to hybridise with in Europe. In areas free from winter frost, which will kill resident maize plants, cultivation and the use of non-selective herbicides may control any subsequent volunteer plants. The risks of spread of the genetic traits are considered minimal.

5.3.2 Treatment of volunteers

Since this maize will not be cultivated in the EC, volunteers in following crops are not a potential problem.

5.3.3 Safety of non-target organisms

In view of the minimal risk of exposure, the fact that this maize will not be grown in the EU and the non-toxicity to vertebrates of any spilled grain, the risk to non-target and beneficial species in the environment from the proposed use of this modified maize is considered remote.

5.3.4 Insect resistance and herbicide tolerance issues

Since this maize will not be cultivated within the EU and is extremely unlikely to escape, potential problems of resistance developing in insect pests and herbicide tolerance developing in non-crop plants are not an issue for the reasons outlined above.

6. OVERALL ASSESSMENT

The Commission requested the Scientific Committee on Plants to consider whether there is any reason to believe that the placing on the market of the T25xMON810 maize for handling and use as any other maize, excluding cultivation, is likely to cause any adverse effects on human health and the environment within the scope of Directive 90/220/EEC. The Committee, after examining the data provided in the dossier and using the background of available knowledge in the areas concerned, considers that there is no evidence to indicate that the import of the T25xMON810 maize is likely to cause adverse effects on human or animal health and the environment within the scope of Directive 90/220/ EEC.

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[1] OJ No L 117, 8. 5. 90, p 15.

[2] click here

[3] OJ No L 131, 5. 5. 98, p.30.

[4] OJ No L 131, 5. 5. 98, p. 32.

[5] OJ No L 043, 14.2. 97 p. 01