Opinion of the Scientific Committee on Plants regarding submission for placing on the market under directive 90/220/EEC of genetically modified processing tomato line TGT7F notified by Zeneca (notification C/ES/96/01) (Opinion of the Scientific Committee on Plants expressed on 23 June 1998)

Opinion of the Scientific Committee on Plants regarding submission for placing on the market of processing tomato line TGT7F with downregulation of polygalacturonase enzyme notified by Zeneca

1. Title

Application for consent to market genetically modified processing tomatoes with downregulation of polygalacturonase enzyme (Notification C/ES/96/01).

2. Terms of reference

The Scientific Committee on Plants is asked to consider whether there is any reason to believe that the placing on the market of the tomato line TGT7F for use as any other processing tomato is likely to cause any adverse effects on human health and the environment.

3. Background

Directive 90/220/EEC 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. For genetically modified plants, the assessment must be based on the information outlined in Annex IIB of Directive 90/220/EEC and take account of the proposed uses of the product.

Following the entry into force of the Regulation on Novel Foods and Novel Food Ingredients (EC No. 258/97) on 15 May 1997, in order for this processing tomato 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.

4. Proposed uses

The products which are the subject of this application are genetically modified processing tomato hybrids derived from the genetically modified inbred parental line TGT7F by conventional breeding methods. The application addresses the production of these tomatoes within the Community, the processing and the products derived from the tomatoes and their eventual use in food (e.g. tomato puree). Tomato processing wastes (seed and skins, known as pomace) are sometimes fed to cattle as a small proportion of their diet (20% maximum). Tomato is thus to be used as any other processing tomato.

5. Description of the product

The product consists of processing tomato (**Lycopersicon esculentum** subsp. Mill.) transformed using the **Agrobacterium tumefaciens** vector system based on the disarmed binary vector Bin19 to introduce the partial sense polygalacturonase (PG) gene isolated from tomato

(**L. esculentum** Mill var. Â‘Ailsa CraigÂ’). The transformation event F leads to the downregulation of the fruit endogenous PG enzyme at the onset of fruit ripening. The only new protein expressed is NPTII (neomycin phosphotransferase) used as a selectable marker. The reduction of PG expression leads to fruit with the potential to extend the harvest window and with modified processing properties.

6. Opinions of the committee

6.1. Molecular/Genetic Aspects

6.1.1. Transformation Technique: Insertion of the genetic material is by **Agrobacterium tumefaciens** -mediated transformation. This method is widely used in plant transformation. The binary vector Bin19 was used.

6.1.2. Vector Constructs: The tomato has been transformed using plasmid vector pJR16S. The plasmid contains between the left and right borders:

(i) a partial sense polygalacturonase (PG) gene from tomato (**Lycopersicon esculentum** Mill) under the control of a 35S promoter from cauliflower mosaic virus (CaMV) and a terminator sequence isolated from the 3Â’ end of the **nos** gene of **Agrobacterium tumefaciens**,

(ii) an **npt**II marker gene from transposon Tn5 from **Escherichia coli** under the control of a **nos** promoter from **Agrobacterium tumefaciens** and a terminator sequence isolated from the 3Â’ end of the **nos** gene of **Agrobacterium tumefaciens**, and

(iii) the following DNA sequence elements; the origin of replication and internal fragment of gene III from bacteriophage M13, two **lac**Z gene fragments from **E. coli** and an **ocd** gene (encoding ornithine cyclodeaminase) from **Agrobacterium tumefaciens**.

6.1.3. Transgenic Construct in the Genetically Modified Plant: One copy of the T-DNA is inserted into the tomato genome. This includes sequences to a partial sense PG gene to co-suppress PG activity, the 35S CaMV promoter, nos terminator, **npt**II marker gene (**E. coli**), nos promoter and the origin of replication (non expressed). PCR shows no insertion of sequences from outside L and R borders. The insert is fully characterised with no functions unknown. The plasmid pJR16S used by the company contains also an M13 gene sequence (gene III) derived from bacteriophage M13. Only a fragment of the M13 gene is present in the plasmid and is not therefore capable of coding for the protein.

6.2. Safety Aspects

6.2.1. Potential for Gene Transfer: The genetically modified tomato contains the antibiotic resistance marker, **npt**II conferring resistance to neomycin/kanamycin, under the control of

nopaline synthase promoter. The company has presented evidence based on PCR techniques that Â‘hot break processingÂ’ used for the preparation of puree denatures and fragments the gene. The limited amount of tomato pomace used as feed for ruminant animals is recovered after such heat processing. Thus the potential for transfer of the **npt**II resistance gene to bacteria of the digestive tract of humans or livestock is essentially zero. In the unlikely event that processing failed or was not applied, it is theoretically possible that this DNA could transform an intestinal bacterium and that recombination could bring the gene under the control of a bacterial promoter. However, even if this occurred, the potential to compromise chemotherapy in humans is negligible. Kanamycin resistant bacteria are relatively common in nature and the introduction of the **npt**II gene would not increase the existing risk to humans to any significant extent.

6.2.2. Safety of Gene Products: Heat processing ensures that the enzyme NPTII does not survive in a biologically active form. Regular human consumption of tomato products containing the heat-denatured protein has not caused recognised problems relating to toxicity or allergenicity. Neither effect would have been expected as judged by comparisons of amino acid sequences made with known antigens, the published lack of effects of the intact NPTII protein in chronic toxicity studies in rats and the recorded ease of degradation of this protein in the digestive tract.

Polygalacturonase is a natural component of all food plants. The truncated PG gene present in the construct is identical to the corresponding endogenous tomato gene and thus confers no additional risks.

6.2.3. Substantial Equivalence: An extensive analysis of fresh fruit and tomato paste samples was made and results compared to the range of values produced by non-modified, commercial tomato varieties. Results for the bulk components (soluble sugars, structural carbohydrate, moisture, ash, fat, carbohydrates including soluble sugars and dietary fibre, protein and oil) and for mineral (Na, K, Ca, Mg, P, Fe) and vitamin (A, E, B_1, B_2, niacin, B_6, folate, C) content fell within the range expected of commercial tomato fruit and fruit purees. Analysis of known anti-nutritional factors and potential toxins (lectins and? -tomatine, solanine, chaconine, tyramine, nicotine and serotonin) demonstrated that a similar range of concentrations of these compounds are present in both modified and unmodified fruit and tomato paste. As a result, the Committee is of the opinion that the harvested genetically modified fruit is substantially equivalent to the fruit of other commercial processing tomato varieties.

6.3. Environmental Aspects

6.3.1. Potential for Gene Transfer/Escape: The cultivated tomato, **Lycopersicon esculentum,** is self-compatible, exclusively inbreeding and essentially cleistogamous (self-pollination and fertilisation occurring within the unopened flower) as a result of unique flower and anther morphology. There are no sexually compatible wild relatives in Europe and feral populations of tomatoes have not become established. Tomatoes do not cross with the closely related **Solanum** genera due to their unique flower morphology. Low frequency crossing between tomato varieties has been demonstrated and attributed to the behaviour and poor foraging activity of insect pollinators. Cherry tomatoes, **L.esculentum** var. **cerasiforme,** do not occur in the wild in Europe but may be grown as protected crops. The chance of genetic exchange outside the crop is therefore small and outside the species remote. The potential

transfer of genetic material to micro-organisms in the soil is considered to be very low against a background of the natural occurrence of kanamycin resistance in soil microbes.

6.3.2. Treatment of Volunteers: Germination of seed may give rise to volunteer plants in the next crop which will be destroyed by normal agricultural practice. Seed dispersal by birds or mammals (by transmission through the gut) away from the crop is insignificant. Due to its tropical origin, the tomato is very sensitive to temperatures below $10^{o}C$ and winter cold will kill the majority of volunteer seedlings following harvest.

6.3.3. Safety to Non-Target Organisms: Tomato flowers are unattractive to insect pollinators due to the limited availability of pollen. Bumble bee activity (**Bombus terrestris**) was found to be no different on modified and non-modified plants. No consistent differences were found during trial releases in the susceptibility of modified and non-modified crops to pests and diseases. No environmental impact on non-target organisms is anticipated.

7. Overall assessment

The Commission requested the Scientific Committee on Plants Â‘to consider whether there is any reason to believe that the placing on the market of the tomato line TGT7F for use as any other processing tomato is likely to cause any adverse effects on human health and the environmentÂ’. In the assessment of the dossier against the criteria set out in Directive 90/220/EEC, the Committee has reached the following conclusion:

- The Committee after examining and considering the existing information and data provided in the Zeneca dossier, against the background of available knowledge in the areas concerned, considers that there is no evidence indicating that the production of the processing tomatoes of Zeneca with downregulated polygalacturonase enzyme and the products derived from the tomatoes and their eventual use as any other processing tomato are likely to cause adverse effects on human or animal health and the environment.