OBJECTIONS TO THE APPLICATION MADE BY THE INSTITUTE FOR WINE BIOTECHNOLOGY, STELLENBOSCH UNIVERSITY

IN RESPECT OF A TRIAL RELEASE APPLICATION FOR GM WINES TSGn and TCGn

TO THE NATIONAL DEPARTMENT OF AGRICULTURE, SOUTH AFRICA

PREPARED BY

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SCIENTIFIC ASSESSMENT

1. THIS APPLICATION

The Department of Viticulture and Oenology in the Institute for Wine Biotechnology (IWB) at the University of Stellenbosch (US) has submitted an application for a trial release of transgenic grapevines to evaluate long-term stability and expression of introduced genes.[i] The focus of the grapevine biotechnology programme at the US is fungal disease resistance and under its auspices, several genetically modified grapevine plants have been developed. The US will make an assessment of substantial equivalence by examining ampelographic, viticultural and vinicultural characteristics. The events in question are of the grapevine cultivars (*Vitis vinifera*) Sultana and Chardonnay and have been designated TSGn (Transgenic Sultana) and TCGn (Transgenic Chardonnay).

The information supplied after a request in terms of the Public Access to Information Act (PAIA) is a limited 39-page copy of the application. Several of the appendices, but most notably Appendix A containing the plasmid map, are blank. Further, the description of the genetic modifications (page 5 of the application) provides no information regarding the characterisation of the transgene, save to list the introduced elements such as promoters, introns, terminators, reporter genes and selectable markers.

In the following discussion, the page references in parentheses refer to the corresponding pages in the application by the Institute for Wine Biotechnology. None of the references cited in the application have been supplied, so an independent reading of this literature cannot be made. It is not a practical option, within the short time period allowed for a response to applications for genetically engineered foods, for respondents to source the referenced literature. We assume that this body of literature forms part of the application as requested in 4.8.3 (page 7) of the application and see no reasonable reason why such information has been withheld.

2. TSGn AND TCGn: DESCRIPTION AND CHARACTERISTICS

Reporter Gene Construct for Events TSGn and TCGn

The reporter gene construct introduced into grapevine cultivars for the development of events TSGn (grapevine cultivar Sultana) and TCGn (grapevine cultivar Chardonnay) has been designated p27GUISC4[2]. As mentioned above, the plasmid map is not included in the documentation received from the Directorate for Genetic Resources in response to a request for Access to information by the African Centre for Biosafety. This construct was introduced into grapevine cultivars by *Agrobacterium*-mediated transformation using biolistic methods. The gene cassette includes a double subterranean clover stunt virus (SCSV) promoter, a *uidA* reporter gene (coding for GUS) with its intron, an octopine synthase terminator, a nopaline synthase promoter and the neomycin posphotransferase

(*nptII*) selectable marker gene.i The sc4 promoter of the subterranean clover stunt virus is a plant-expressible promoter i.e., a DNA sequence, in this case of viral origin, which is capable of controlling (initiating) transcription in a plant cell.[ii]

The *uidA* gene codes for β-glucuronidase (GUS), an enzyme from the bacterium *Escherichia coli*.[iii] Promoter expression can be assessed because the GUS enzyme converts a colourless substrate to a clear blue colour. GUS assays are useful in higher plants because of the lack of any detectable GUS activity in these organisms. The GUS reporter system is in this case being applied to a determination of the success of the genetic modification by serving as a visual marker for modified tissues.i

Antibiotic Resistance Markers

Antibiotic resistance marker genes are used often in the development of transgenic crops as selectable markers. Selectable markers allow the modified form to be selectively amplified while unmodified forms are eliminated. The use of antibiotic resistance markers has application in development of the transgenic line allowing for selection of modified plants in the laboratory. The transgenic crop line however, will retain the marker gene for its lifetime in each of its cells.

The *nptII* gene from *Escherichia coli* expresses the enzyme neomycin phosphotransferase II (NPTII), which inactivates principally kanamycin, geneticin and neomycin by phosphorylation, that is used to select transformed cells.

Imprecision of Plant Modification Techniques and Possible Consequences

The lack of molecular characterisation information makes an assessment of the expression of the introduced gene sequences nigh impossible. The IWB claim that gene stability of the transgenes has "scientifically been proven" (pg 2) under greenhouse conditions. No evidence is provided to support this statement.

Unintended effects that are not detected in the lab and that may only become apparent in the long term, cannot be ruled out. Transformation by particle acceleration is associated with multiple fragments and gene rearrangements.[iv,v] Inserted gene sequences may interrupt native gene sequences and/or their promoters and additional code fragments are not necessarily non-functional and may be transcribed. Extra gene fragments in Monsanto's Roundup Ready Soya were also claimed to be non-functional and not-transcribed,[vi] but were later found to be transcribed to produce RNA.[vii,viii.]

Further, it is not clear if the insert or fragments thereof lie on any transposons and what the impact of the DNA insert is on flanking sequences. The lack of sophisticated methods for targeted insertion, especially in higher organismsv necessitates more rigorous research into possible position effects prior to the granting of any release of transgenic organisms into the environment. Further, if transgenes behave just like naturally occurring genes, then they have the potential to be inherited in the same way and persist indefinitely in cultivated or free-living populations. Any mixing of native and

transgenic plants whether by dispersal, improper handling etc., can result in the spread of transgenes. The consequences, both ecological and evolutionary of crop-to-crop gene flow are only now beginning to be investigated in any meaningful way and the possible exposure of non-target organisms, including humans to novel proteins cannot be discounted.v

3. TRANSFERRED ANTIBIOTIC RESISTANCE GENE AND THE SAFETY THEREOF/HORIZONTAL GENE TRANSFER

Horizontal Gene Transfer (HGT)

Horizontal gene transfer (HGT) is the transfer of genetic material between organisms, outside the context of parent to offspring reproduction[ix],[x]. It is most commonly recognized as infectious transfer[xi]. HGT frequencies are now known to be much higher than originally thought. The evolution of antibiotic resistance, for example, is an indicator of the frequency of gene transfer, given that antibiotics have been used in medicine only for about 50 yearsxi. The intentional modification of plants could through horizontal gene transfer result in the unintentional modification of other organisms. What the possible impacts of such gene transfer might be is not known.

Potential for HGT of Antibiotic Resistance Marker Genes (ARMG)

The significance of any potential gene transfer is dependent on the marker being transferred and what its existing or future therapeutic application is or might be. Where there are antibiotic resistant marker genes, as in TSGn and TCGn (*nptII*), there is a potential for gene transfer of these markers to pathogenic organisms. The encoded product inactivates aminoglycoside antibiotics such as kanamycin and neomycin. Kanamycin, contrary to popular belief is still used in medical applications, e.g. prior to endoscopy of the colon and rectum[xii] and to treat ocular infections[xiii]. It is well known that there is cross resistance between antibiotics of a particular typex. Neomycin was found to cross react with kanamycin B in inhibiting RNAse P ribozyme 16s ribosomal RNA and tRNA maturation[xiv]. Other aminoglycoside antibiotics including streptomycin, gentamycin and tobramycin, which are used to treat human disease, have exhibited cross resistancex. The possibility of transfer of the marker by HGT, and subsequent adverse effects on human and animal health, cannot be ruled out in those cases where these antibiotics are still being used.

Several European countries including Austria, Luxembourg, France, Norway and the United Kingdom have expressed grave concerns about the presence of antibiotic genes in GM products and the EU has as a result, decided to prohibit GMOs with antibiotic resistance genes after the 31st December 2004 (directive 2001/18EC and Revising Directive 90/220/CEE)[xv]

4. OUTCROSSING AND GENE TRANSFER

Pollination

Grapevine is largely self-pollinating with insects rarely playing a role due to due to the form of the flower and limited nectar. Sultana is a seedless cultivar which does not produce viable seeds, therefore, self-pollination of the transgenic grapevines, or fertilisation of other Sultana grapevines by pollen from the transgenic plants, would result in seedless fruit. There is a small possibility that air-borne pollen from the transgenic plants may be carried to other vines under cultivation on the site. The pollen flow study referenced by the applicant (page 6) was headed up by Director Reinhard Töpfer of the Institute for Vine Breeding (IVB) Geilweilerhof. The IVB has announced in 2005 that no research on pollen dispersal in vine has been undertaken so far - apart from their work in the context of the German programme for biological safety. The main findings were that that GE pollen could be detected within 100m, no pollen was detectable between 150-400m, and outcrossing of 2,7% could be detected in 20m in the dominant wind direction. These trials with genetically engineered vines in Germany have been stopped prematurely by the IVB because the varieties which had been genetically engineered to possess resistances against certain fungal pests appear to be as susceptible as conventional vines. "With regard to fungal resistance no advantage could be seen with the genetically engineered vines compared to the controls," said Prof. Topfer. The trials were also meant to address questions of Biosafety and we surmise that the suspension of the trials means the end of the Biosafety testing into these varieties.

Further, volunteer plants may arise should pollen from the transgenic plants fertilise adjacent grapevines of fertile varieties. The Chardonnay berries contain 2-4 seeds per berry and seed dispersal is possible by humans and animals, notably birds. There is the possibility that animal exposure might occur especially after rain and storms where grape berries could drop to the ground and escape from the site in rain water.

Occupational Exposure

There is a small possibility that air-borne pollen from the transgenic plants may be carried to other vines under cultivation on the site. Human intervention means that some fruit may be collected. There is also a level of exposure to the GM grapevines through inhaling the pollen[xvi] and certain individuals may be allergic to grape fruit or grape pollen. No toxicity or allergenicity information on these two events has been provided.

5. ALLERGENICITY

The nature of genetic modification of higher plants results in the production of novel proteins which might cause allergic reactions. Allergies to food are potentially life threatening for an estimated 2% of adults and 8% of children. One reason for the failure of identification of GM crops as allergenic is related to the fact that the testing and assessment thereof is left up to the developer of the transgenic organism and that no

standardised agreed-upon protocols exist for such testing.[xvii] No test exists that is fully predictive of potential allergenicity.[xviii]

The need for the assessment of allergenicity was first recognised when Pioneer transferred Brazil nut genes for a high methionine 2S albumin into soybeans and detected its allergenic potential and voluntarily stopped development of the product.[xix,xviii] This highlighted the need for a sound assessment strategy for allergenicity and over the past ten years, several bodies have applied themselves to this including the International Life Sciences Institute, the International Food Biotechnology Council, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).xviii[xx]

Assessment of Allergenicity

Regulatory authorities considered several elements for testing including the source of the gene, sequence homology to known allergens, specific serum screening, comparative resistance to pepsin, target serum screening (the immunoreactivity of the novel protein with serum IgE from individuals with known allergies to species that are broadly related to the source of the transferred DNA) and the use of animal models. The latter two methods were not considered sufficiently well understood or developed methodologies for regulatory purposes and to date, the allergenicity assessment of genetically modified food crops relies on the four former-mentioned methodsxviii.

The gastric stability assay has been widely accepted as an important part of allergenicity assessments of genetically modified products and support in the literature continuing through the FAO/WHO consultation in 2001 resulted in acceptance by the Codex Alimentarius.[xxi,xxii,xxiii] This experiment is based on the hypothesis that food allergens must exhibit sufficient gastric stability to have a chance of reaching the intestinal mucosa where absorption and sensitising will occur.xviii[xxiv] Typically the test is a measure of comparative resistance to pepsin proteolysis.xviii In the face of the lack of definitive tests for determining potential allergenicity, it is the most reliable test.xxiii[xviii,xxv] The potential for allergenicity of the GE grapevines is "very low or negligible" because 'the introduced proteins are already present in the natural environment" (page 18). This statement cannot be accepted on the face of it and we are not in any position to make an informed assessment due to the lack of molecular characterisation data, allergenicity or toxicity data. The introduction of DN A sequences by biolistic transformation could disrupt any of the cellular processes in which DNA or RNA participate, including replication, transcription, translation, recombination, transposition. The level of expression or the timing of the expression of any protein that is normally expressed in a food-producing organism, the allergenicity or toxicity of the food derived from that organism as well as the nutritional characteristics of the food may be altered.[xxvi]

6. WEEDINESS

Whilst no wild relatives of the grapevine may be found in South Africa, (page 13), the genetic modifications to the grapevine have resulted in GM grapevines that differ from conventional grapevines in that antibiotic resistance proteins are expressed. These new proteins/enzymes may alter plant characteristics and functions and may have an impact on the weediness of the grapevines.xvi

7. GENETIC MODIFICATION: DEGREE OF CERTAINTY

In general, genetic modification by the application of recombinant DNA technology is characterised by scientific uncertainty. This stems from several factors including the inherent imprecision of currently employed recombinant DNA techniques, the use of powerful, often viral, promoter sequences in genetic constructs and the generation, as a result of genetic modification, of novel proteins to which humans and animals have never previously been exposed[xxvii]. Additionally, the gaps in the knowledge regarding composition and functioning of the genomes that are often subjected to genetic manipulation and ill-designed experiments compound such scientific uncertainty.xxvii

Uncertainty is a key element of the Biosafety Protocol (Cartagena Protocol on Biosafety to the Convention on Biological Diversity).[xxviii] The lack of sufficient relevant scientific information and knowledge regarding the extent of potential adverse effects allows the Precautionary Principle referenced in the Biosafety Protocol to be triggered. The precautionary principle states that "where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be use as a reason for postponing cost-effective measures to prevent environmental degradation".

8. CONCLUSIONS-SCIENTIFIC ASSESSMENT

The available scientific information, as provided by the applicant, does not allow for a full evaluation or determination of the associated risks of the use of the transgenic lines. At a minimum, the literature indicates that a great deal more investigation has to be carried out on the impacts of transgenes before their release into the environment. The potential hazardous or deleterious effects resulting from the trial release as postulated by the applicant include "toxicity or allergenicity to humans and other organisms, weediness and transfer of introduced genes to other organisms" (page 11).

None of the results of the gene stability studies have been provided (page 2). The ultimate aim of the modified grapes is for use as food and wine will be made from the Chardonnay grapes (page 11)No indication is given of what the future intention of the transgenic development is and the claimed purpose of the trial is as being for 'proof of concept" only. No assessment can be made of possible differences between the native and genetically modified form due to a lack of characterisation information by the applicant.

Any potential category of risk introduced by the genetic modification as compared to risks from conventional breeding is still unclear from the application. The ability of ecosystems to develop gradually, the ability to anticipate environmental health effects and very importantly, the establishment of regulatory mechanisms that can effectively, efficiently and credibly manage risks associated with the use of genetically modified organisms (GMOs) has not kept apace with the rapid introduction of GMOs. Traditional breeding practices have an established history of safe use dating back several years as opposed to the application of recombinant DNA technology for human use, which is as young as 22 years when genetically modified bacteria-produced insulin was first introduced and even younger for genetically modified plants at ten years.xxvii In summary then:

- A full assessment of the scientific data could not be made because of the withholding of key appendices and molecular characterisation information

- Genetic modification by the application of recombinant DNA technology is characterised by scientific uncertainty. This stems from several factors including the inherent imprecision of currently employed recombinant DNA techniques, the use of powerful promoter sequences in genetic constructs and the generation, as a result of genetic modification, of novel proteins to which humans and animals have never previously been exposed

- No toxicity or allergenicity information is available and no studies have been conducted by the applicant to this end

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