OBJECTION TO APPLICATIONS MADE BY THE SOUTH AFRICAN SUGARCANE RESEARCH INSTITUTE (SASRI) FOR PERMITS FOR TRIAL RELEASES OF GENETICALLY MODIFIED ORGANISMS (GMOs) INTO THE ENVIRONMENT OF SOUTH AFRICA

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INTRODUCTION

An assessment was made of the information obtained from the National Department of Agriculture, Forestry and Fisheries (DAFF), in terms of the Public Access to Information Act (PAIA), of the South African Sugarcane Research Institute's (SASRI) applications for trial releases of GMOs The information supplied by the applicant (non-confidential biotechnological information) is very scant and does not allow for a full and fair public participatory process.

BACKGROUND

APPLICATIONS BY SASRI AND AVAILABLE INFORMATION

Applications have been made by the South African Sugarcane Research Institute (SASRI) for trial releases of genetically modified sugarcane lines of the variety NCo310. Four applications have been made in which:

1. Sucrose content has been increased through the down-regulation of a gene involved in nucleotide synthesis and turnover (designated "**A**"); the activity of Uridine Monophosphate Synthase has been down-regulated

2. Starch content has been decreased by the down-regulation of a gene in the starch biosynthetic pathway (designated "**B"**), the change being effected through down regulating the activity of ADP-Glucose Pyrophosphorylase.

3. Starch content has been increased by the down regulation of a gene mediating the synthesis and turnover of purines, and (designated "**C**"), specifically impacting on the activity of adenine dinucleotide kinase. This line with increased starch content is stated by SASRI to represent a potential alternative for use in biofuels production.

4. The content of a cell wall constituent has been increased by the expression of non-sugarcane gene encoding the specific cell wall component (designated "**D**"). The activity of cellulose synthase has been increased with the aim of increasing cellulose content with the aim of producing a line for bioethanol production.

The information supplied after a request in terms of the Public Access to Information Act (PAIA) is copies of the applications (Non-confidential biotechnological information) and copies of the Public Notices.

It is understood that for all of the lines, that plantings will occur in containers placed on a concrete terrace. **A** will additionally be evaluated for crop performance in a designated field.

THE HOST PLANT AND MODIFIED SUGARCANE VARIETIES

Sugarcane, a perennial grass with no single genetic origin, consists of six species – two wild species, *S. spontaneum* L. and *S. robustum* and four cultivated species, *S.officinarum* L., *S. barberi* Jeswiet, *S. sinense* Roxb and *S. edule.* Hassk.[1] By and large, sugarcane is vegetatively propagated and does not depend on seeds. What is sold to farmers and afterwards planted is sections of the cane with shoot buds. At the time of harvesting, the roots are left in the soil for regeneration of new canes. It is necessary to plant with new buds every four years.

GENETIC TRANSFORMATION EVENTS

For **A**, **B**, **C** and **D** two plasmid vectors were used:

The construct pEmuKN contains the bacterial selectable marker gene *nptII*, gene from *Escherichia coli* which was used as a selectable marker plasmid for all transformations, conferring resistance to the antibiotic geneticin.

The co-transformation construct contains the maize ubiquitin promoter, cauliflower mosaic virus (CaMV 35S) promoter, exon, and intron, the gene of interest and the CaMV termination sequence. This construct, in each case, uses ampicillin resistance as a selectable marker.

No plasmid maps and scant assessment information is provided in each of the applications. There is no way of assessing:

- The region intended for insertion
- Whether the probes used provided complete coverage of the rDNA (recombinant DNA) including that which was and was not intended for insertion
- Whether or not the plasmid backbone was not integrated into the genome of the GMO
- whether the probes used were of an acceptable sensitivity
- What the minimum size target is, that each probe would detect at a maximum of 0.5 copies per genome at the stringency used in the Southern blots. Without this information it is impossible to exclude other inserts and inserts of vector DNA
- whether smaller probes were used

Large probes will adhere well to full or near full length insertions allowing the applicant to use very high stringency washes of the Southern blots. The high stringency washes reduce the chances of "false positive" associations between probe and genomic DNA. However, for a biosafety assessment, false negatives are far more important. Small or rearranged inserts with fewer matches to the large probes will be "washed off" at high stringency and thus would be missed.

The sensitivity of analysis of genomes for insertions of partial rDNA fragments must be at least to the standard of published studies that have been able to demonstrate much higher effectiveness at detecting unexpected inserts.[2,3,11] It is recommended that the developer provide the complete sequence of the rDNA. Information on modifications that affect the final amino acid sequence of the product of any transgene also should be provided.

INADEQUACY OF INFORMATION SUPPLIED BY APPLICANT

The ACB has received an astonishing paucity of information – about 35 pages of information for each application (**A**, **B**, **C** and **D)**, with the result that it has been severely hamstrung in conducting any meaningful assessment of the applications. This biased and grossly inequitable situation has arisen principally, because the DAFF has failed to establish a proper formal process for the determination and characterisation of what constitutes confidential business information (CBI). This assignation is left entirely to the discretion of the applicant, in this case SASRI, and has resulted in the ACB being severely prejudiced in objecting to this application.

CONCERNS REGARDING GENETIC MODIFICATION

From the little information that has been provided on the constructs developed, we record our concerns below.

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 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[4]. 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.[4]

Uncertainty is a key element of the Biosafety Protocol (Cartagena Protocol on Biosafety to the Convention on Biological Diversity.[5] 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 “where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation”.

POSSIBLE UNINTENDED EFFECTS OF THE NON-FUNCTIONAL DNA FRAGMENTS

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.[6,7] The European Commission Scientific Committee on Food[8] has stated that the lack of transcription or translation signals from Northern and Western blots, does not 'preclude absolutely the possibility that the truncated gene is expressed but the possibility that this is the case will be extremely remote.'[8] 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[9], but were later found to be transcribed to produce RNA.[10,11]

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 organisms,[7] 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.[7]

STABILITY OF THE CAMV PROMOTER

The genes in **A**, **B**, **C** and **D** are under the control of the Cauliflower Mosaic Virus CaMV35S promoter and terminator. The CaMV 35S promoter has been found to have a recombination hotspot where it tends to fragment and join with other double stranded DNA in very non-specific way.[12] These hotspots are flanked by multiple motifs involved in recombination and functions efficiently in all plants, green algae, yeast and *Escherichia coli*. The potential exists for the viral genes to recombine with other viruses to generate new infectious viruses,[12] carcinogens and mutagens and reactivate dormant viruses. Detractors claimed that virus infected cabbages and cauliflower have been consumed for years with no ill effects and that similar pararetroviral sequences occur widely in plants causing no apparent harm.[13] That the intact virus causes no obvious harm in the natural host is related to the fact that its integrity is maintained and that it is adaptive to the host biology. This is unlike the fragments of naked DNA as in transformed plants where the natural regulatory mechanisms are not present.[12] A call has been made that the use of the CaMV promoter in transgenic plants be phased out due to the structural instability arising out of its use.[14] Information relating to "event specific" molecular analysis has not been provided for any of the transgenic events.

We believe it to be necessary that such molecular characterization be carried out and submitted or if it has been carried out be made available for independent scrutiny.

SELECTABLE MARKER GENES

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.[15]

The use of antibiotic resistance genes in transgenic organisms has raised human health concerns because the combination of resistance genes in food and potential bacterial pathogens in the gut could create more opportunity for antibiotic resistant disease-causing bacteria to arise. In one study about the survival of transgenic DNA in a human feeding study, the transgene could be detected from isolated gut bacteria, albeit in low concentrations and after enrichment culture.[16,17] There are multiple well-known mechanisms for cross-resistance to antibiotics of a particular type.[18]

HORIZONTAL GENE TRANSFER (HGT)

Horizontal gene transfer (HGT) is the transfer of genetic material between organisms, outside the context of parent to offspring reproduction.[19] It is most commonly recognized as infectious transfer.[20] 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 years.[20] 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 such as *nptII*, there is a potential for gene transfer of these markers to pathogenic organisms. Geneticin is toxic to bacteria, yeast, protozoa, helminths, and mammalian cells.[21] Ampicillin is widely used for treatment of human bacterial infections and its spread to harmful organisms could compromise its therapeutic value. 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).[22]

ENERGY BALANCE AND DEVELOPMENT OF FOOD CROPS FOR BIOFUELS

Agrofuels, also known as biofuels are fuels made from plants and animal fat. Since they are not derived from fossil sources like coal or oil, proponents claim that they can help mitigate global warming. Motor vehicle emissions are responsible for 14% of global warming.

A UN report "Sustainable Bioenergy: A Framework for Decision Makers" released in 2007,[23] found that agrofuels are the fastest growing sector in world agriculture. The *Financial Times* estimates that OECD country subsidies for agrofuels amount to a total of $15 billion dollars a year. The industry expected production to increase from 11 billion gallons in 2006 to 87 billion by 2020, and the market to grow from $20.5 billion in 2006 to $80.9 billion in 2016.[24]

At the beginning of 2006, South Africa phased out the use of lead, which created a boon to the ethanol industry, as ethanol can be used as an additive to boost the octane number of unleaded fuel. In addition, and following on from the lead of the US, at the launch of the National Energy Regulator of South Africa in November 2005, Deputy President Phumzile Mlambo-Ngcuka said that the South African Cabinet had approved a proposal by the Departments of Minerals and Energy (DME), Agriculture and Land Affairs, and Science and Technology, to explore biofuels as an important component of South Africa's energy mix. Touted as a cleaner, greener fuel, by reducing CO_2 emissions by 60%, ethanol is said to bring huge socio-economic benefits through especially job creation.

The claimed positive effect on reduction of CO_2 emissions from biofuel production is in question as well. In a study by Grain[25] it was reported that "Recent studies have shown that the production of one tonne of palm-oil biodiesel from peatlands in South-east Asia creates 2–8 times more CO_2 than is emitted by burning 1 tonne of fossil-fuel diesel".[26]

The energy balance for the production of biofuels is also skewed. A study by Pimental and Patzek shows that turning plants such as maize, soyabeans and sunflowers into fuel uses more energy than the resulting ethanol or biodiesal generate.[27] The researchers demonstrated that sunflower oil requires 118% more fossil energy to refine it than the fuel obtained from it. Likewise, soya requires 27%, and maize 29% more fossil fuels than that obtained from the crops themselves.[27]

A recent study found that the bio-energy potential of Sub-Saharan Africa-after accounting for food production and resource constraints-was the greatest among all major world regions.[28] The high potential results from the large areas of suitable cropland in the region,

large areas of pasture land that are not currently used and the low productivity of existing agricultural production systems as well as the low cost of labour. South Africa's total land area is 121.4 Mha, of which, 99.6 Mha or 82% is comprised of total share of agricultural area, of which only 12.9 % is comprised of cultivated area.[29] There is concern that land currently used or with the potential for use for food production will be given over to crops intended to be turned into biofuels.

For instance in the Eastern Cape, the Provincial Biofuels Task Team and Eastern Cape Development Corporation, revealed plans to plant canola on 500,000ha of the most arable non-irrigated commonage and communal land in the former Transkei and then process it into bio-fuel at a plant in the East London industrial development zone. R1.5 billion will be spent on fencing and liming this land to prepare it for monoculture. Furthermore, while local communities forego their existing diverse food gardens and communal grazing lands, multinational companies like Monsanto will collect on government agricultural subsidies through the Massive Food Production Programme by providing seed, chemical inputs and even mechanisation on the farmer's behalf.[30] The EC Premier's State of the Province Address for 2007 confirms that an initial 70,000 ha of irrigated land in the Umzimvubu valley was to be placed under canola monoculture in the next season for biofuel production.[31,32,33]

CONCLUSIONS

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. 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. The impression gained from the notifiers responses is that any possible impacts of the release of the transgene are negligible and that the transgenic line is equivalent to the conventional type – this is a view not supported by the published literature.

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 applicant has made claims of no adverse effects to human and animal health and the environment from release of the transgenic organism the reason is given that there is no difference between the native and genetically modified form. The preceding discussion makes it clear that this is not the case. At the very least, independently verifiable research has to be carried out before such claims are made.

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 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.[4]

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