AFRICAN CENTRE FOR BIOSAFETY

OBJECTIONS TO THE APPLICATION MADE BY MONSANTO SOUTH AFRICA FOR A COMMODITY IMPORT PERMIT OF GRAIN FOR FEED AND FOOD PURPOSES THAT MAY CONTAIN MAIZE GRAINS DERIVED FROM INSECT-PROTECTED MAIZE LINE MON863 AND MAIZE HYBRIDS MON863 X MON810

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SCIENTIFIC OBJECTIONS

A scientific assessment was made of the available information. The main findings of this assessment, which are discussed in greater detail later in the document, are:

— A full assessment of the scientific data could not be made because of the designation of large sections of this data as Confidential Business Information

— The molecular characterisation information provided by the notifier indicates several irregularities including open reading frames, a missing stop codon and truncated constructs which could give rise to unintended gene effects

— There is evidence of structural instability arising out of the use of the 35S CaMV promoter and there is a call for the discontinuation of the use of this genetic element in the development of transgenic plants

— The protocols for assessing gene expression in transgenic plants are flawed. Nevertheless, a consideration of the gene expression data provided shows elevated levels of the expressed protein relative to the wild type

— Lack of allergenicity in Cry3B sprays cannot be taken as confirmation that the same applies for the Cry3Bb1 protein due to molecular differences in the transgenic form

— The tests to determine allergenicity have been incorrectly applied (sequence homology assessments), not correlative (digestion studies) and inadequate (several tests not conducted)

— Horizontal gene transfer frequencies are much higher than previously thought and given the presence of an antibiotic resistance marker gene, there is potential for antibiotic resistance transfer to other species

— The decision by the EU to prohibit the use of antibiotic resistance markers in GMOs raises concern about the presence of such a marker in these lines

— Feeding studies do not supply adequate information for an independent assessment of their validity

— The hybrid should be fully assessed as a genetically engineered organism separate from MON810 and MON863

SUMMARY OF LEGAL ISSUES

1. Monsanto has not discharged its onus of proving that its genetically modified (GM) maize is safe for human and animal consumption, as it has failed to submit adequate scientific data for consideration under the GMO Act and thus, it is not possible for the Executive Council to make any reliable safety assessment. Indeed, taking into account our scientific assessment, it is our respectful submission that the Executive Council must

refuse the application, on the basis of the precautionary principle as reflected in Article 11(8) of the Cartagena Protocol on Biosafety. The precautionary principle demands a rigorous scientific approach and ensures democratic decision-making in regard to the acceptance of risks. It also requires the seeking and considering of sustainable alternatives precisely because it explicitly considers uncertainty and ignorance.

2. The Constitution of the Republic of South Africa obliges the State to ensure that South Africans have the right to safe food-as a critically import socio-economic right. Maize is a critically important agricultural product because it is used as a staple for millions of people not only in South Africa, but also in the Southern African region.

It is our submission, taking into account our scientific assessment of the application; that the right to safe food enshrined in the Constitution will be flouted should the Executive Council grant the approval sought by Monsanto. Indeed, the Department of Health has a constitutional and statutory duty to safeguard the consumer from foodstuffs that are harmful or injurious to human health as is also borne out by the rationale for the Foodstuffs, Cosmetics and Disinfectants Act No 54 of 1972.

3. The National Department of Agriculture has approved Monsanto's MON 810 for commercial growing and import as food and feed. It has also approved Monsanto's GM maize event 863 for import as food and feed. Yet, neither it, nor the Department of Health nor any other government agency has to date, conducted any reliable and proper post commercialisation testing and monitoring for the effects of transgenic maize on animal and human health. Taking into account our scientific assessment, and our submissions below regarding post-commercialisation testing and monitoring, the South African government is entitled to review its earlier decisions taken regarding safety approvals given by it, in respect of Monsanto's GM maize MON 810 and transformation event 863, in terms of Articles 12 and 11(8) of the Biosafety Protocol.

4. The Department of Trade and Industry, the NDA and the Department of Labour should, as a matter of extreme urgency, conduct an assessment on the socio-economic impacts of the importation of GM maize in the hundreds of thousands of metric tons by the animal feed industry in South Africa, from Argentina and the United States. This assessment must include an enquiry into impacts on the domestic production of maize in South Africa, the distortions in the market place caused by the sale of such maize, the long-term food security and food sovereignty impacts for South and Southern Africa, the predatory pricing policies of international grain exporters such as Cargill and Louise Dreyfus and the huge subsidy regimes available to them by their governments that assist them in obtaining market domination and displacement of local producers and placing at risk, thousands of jobs in the agricultural sector and related industries.

5. We are extremely concerned about the negative environmental impacts that may arise from the spillage of whole GM maize grains during transportation and the milling process itself. We note with alarm that the transportation of GMOs as well as the mills to

be used in the processing of GMOs is captured by the extraordinarily wide definition of contained use in section 1 the GMO Act. We are aware that the Registrar, Dr Julian Jaftha has recently proposed five measures regarding the importation of genetically modified maize that have only commodity clearance in South Africa. Whilst these measures are a welcome step towards greater biosafety, until such time as they are brought within the purview of the GMO Act, the NDA will not have the legal powers to ensure enforcement and compliance. We also point out, that we are not convinced that any proper monitoring has or will take place, to ensure that GMOs imported for food and feed does not cause harm to the environment as a result of spillage during import, transport and processing phases. In fact, we are not aware of any measures being taken by either the NDA or the Department of Environmental Affairs and Tourism of such monitoring.

LEGAL ISSUES

1. THE RIGHT TO SAFE FOOD

The Constitution of the Republic of South Africa 108 of 1996 is the highest law. The supremacy clause in the Constitution is contained in section 2 which provides:

" This Constitution is the supreme law of the Republic; law or conduct inconsistent with it is invalid; and the duties imposed by it must be performed."

The introduction of the interim Constitution and the final Constitution marked a decisive break with the past. The Constitution is not neutral on fundamental values. The Constitution contains a vision for the transformation of society. The centrality of the Bill of Rights and its foundational values to the newly created democracy is expressed in section 7 of the Constitution, which provides:

"Rights

7 (1) This Bill of Rights is a cornerstone of democracy in South Africa. It enshrines the rights of all people in our country and affirms the democratic values of human dignity, equality and freedom.

(2) The State must respect, protect, promote and fulfil the rights in the Bill of Rights.

(3) ……"

Section 27 of the Constitution forms part of the cluster of socio-economic rights dealing with the right to health care, food, water and social security. These rights, read together with the provisions of section 24 of the Constitution entrenches amongst others, the rights of all South Africans to an environment that is not harmful to health or well-being. It imposes a duty on the state to protect the environment, for the benefit of present and future generations.

The Constitution implicitly obliges the State to ensure that South Africans have the right to safe food-as a critically import socio-economic right. Maize is a critically important agricultural product because it is used as a staple for millions of people not only in South Africa, but also in the Southern African region.[1] Section 27 provides that: "(1) Everyone has the right to have access to –(a) …… (b) sufficient food and water; and ….." Implicit in the right to access to food is the right to expect that such food and water is safe for human consumption. Section 27(2) requires the State to take "reasonable legislative and other measures" to achieve such rights. It cannot simply sit back; it must take active measures. The Constitutional Court has delivered two important decisions on the ambit and justiciability of socio-economic rights:

- Government of the Republic of South Africa and Others v Grootboom and Others 2001 (1) SA 46 (CC)
- Minister of Health and Others v Treatment Action Campaign and Others (No.2) 2002 (5) SA 721 (CC)

It is our submission, taking into account our scientific assessment of the application and the lack of monitoring by the government of the impacts of GM maize on animal and human health, the right to safe food enshrined in the Constitution, that the Executive Council will flout these constitutional rights should it grant the approval sought by Monsanto. Indeed, the Department of Health, who plays an oversight role on the Executive Council in terms of the GMO Act, has on obligation to safeguard the consumer from foodstuffs that are harmful or injurious to human health. This general obligation is also created by the Foodstuffs, Cosmetics and Disinfectants Act (No 54 of 1972).

2. LEGISLATIVE LACUNA: POST COMMERCIALISATION TESTING AND MONITORING FOR THE EFFECTS OF TRANSGENIC FOOD AND FEED

The National Department of Agriculture has approved Monsanto's MON 810 for commercial growing and import as food and feed. It has also approved Monsanto's GM maize event 863 for import as food and feed. Yet, since the time when these GMOs were approved, neither the NDA, nor the National Department of Health nor any other government agency has conducted any post commercialisation testing and monitoring for the effects of transgenic maize on animal and human health. This failure has arisen

because the GMO Act does not address the issue of post commercialisation testing and monitoring adequately or at all.

The GM maize in question, also referred to as 'yellow maize' is used in South Africa as an ingredient in feed rations for diary, beef, poultry and egg production.[2] This maize is also a raw material for the production of starch used in turn, in the manufacture of sweeteners, syrups, and fermentation products. Maize oil is also extracted from the germ of the kernel. Thus maize products are present in a wide range of processed food products.

Neither Monsanto, the Department of Health nor the Executive Council are in any position to make the assumption that Monsanto's GM maize, MON810 and 863 are safe for human and animal consumption, "because no one has become ill or died as a result of consuming the GM maize" as is so frequently stated. This is particularly pertinent, given that South African legislation does not require the labelling of GM food and feed, and hence authorities in South Africa have no way of monitoring what and how much of GM food and feed has been consumed over any given period of time.

Rationale for Monitoring

The reasons for post commercialisation testing and monitoring include *inter alia,* the following:

(a) To determine if pre-commercialisation testing protocols adequately assess the risks;
(b) Long term monitoring is needed to record trends in predicted effects and to detect effects which were not predicted;
(c) Post-commercialisation testing or validation is part of quality control;
(d) Evidence collected over a period of time can confirm the accuracy of pre-release protocols;
(e) Low probability and low magnitude effects would likely escape detection in test-experiments;
(f) To observe smaller and less frequent health risks, an appropriately long time scale is needed;
(g) Rigorous monitoring reassures the public; and the NDA and DOH cannot continue to ignore public health concerns, to do so is irresponsible;
(h) Pre-commercialisation risk analysis has several weaknesses: small scale experiments are only capable of detecting large effects (order of magnitude differences); and
(i) Different kings of monitoring are required for different needs;

Recommendations

The GMO Act must be urgently amended to include comprehensive provisions dealing with the testing and monitoring of the impacts on the environment, animal and human

health of GM food, feed and plants. In regard to the testing and monitoring of GM food and feed, the following preliminary recommendations are made:

Animal Health Monitoring should include inter alia, the following:

- Growth and life span: organ development;
- Disease susceptibility: immune status, pathogenicity, infectiousness; and
- Reproductive function-these should take place over at least 4 generations.
- Short and long term monitoring of animal behaviour: health, physiology and metabolism;

Monitoring of Humans

There is a range of techniques that could be used for this purpose. These include non-invasive techniques such as testing immune responsiveness, consecutive blood sampling, hormone assays, and bacterial status etc.

Invasive techniques could include gastric biopsies, tumour histology, and pathology testing. Testing and monitoring can also take place by using human volunteer studies and in this regard, new microbes (viruses, bacteria) containing GM vector elements should be monitored. Particular attention must be paid to the identification and monitoring and invasion of bacteria with antibiotic resistant genes.

3. PRECAUTIONARY PRINCIPLE AND THE BIOSAFETY PROTOCOL

South Africa is a Party to the Biosafety Protocol, it having ratified the Biosafety Protocol on the 14 August 2003. The Biosafety Protocol became binding on South Africa on the 12 November 2003. In terms of Section 231 of the Constitution of the Republic of South Africa, 1996, an international agreement such as the Biosafety Protocol is binding on South Africa.

In terms of the Biosafety Protocol, South Africa as a Party is entitled to take decisions regarding the import of GM maize for food, feed and processing on the basis of the precautionary principle as set out in Article 11(8) of the Protocol, which provides as follows:

"*the lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a LMO on biodiversity, taking into account risks to human health, shall not prevent a Party of import from taking a decision, as appropriate, with regard to the import of the LMO in question.*"

These provisions of the Protocol are seen to represent the most explicit examples of operationalisation of the precautionary principle/approach in any multilateral environmental agreement.[3]. As has also been canvassed elsewhere in this submission, it is our submission that, having regard to our objections, South Africa should reject the application by invoking Article 11(8) of the Biosafety Protocol.

4. REVIEW OF DECISION AND THE PRECAUTIONARY PRINCIPLE

As a Party to the Biosafety Protocol, the South African government is entitled to review earlier decisions taken regarding the commodity clearance given by it with respect to Monsanto's GM maize Mon 810 and transformation event 863, as contemplated by Articles 12 and 11(8) of the Biosafety Protocol.

Article 12 of the Protocol allows South Africa to review its decision on imports of GMOs in the light of new information or circumstances about the risks to the environment, biodiversity and human health i.e. scientific information regarding the negative impacts of the GM maize in question, on human health that may not have existed or may not have been known by the South African government at the time a decision was taken.

It is clear from our objections that the information and circumstances as contemplated by Article 12 of the Biosafety Protocol now clearly exist. It is therefore incumbent upon the South African government, as a Party to the Protocol, to review its prior approvals for the GM maize events in question (MON810 and 863).

Moreover, it is imperative that the government review its decision, based on the precautionary principle set out in Article 11(8) of the Protocol. We have already canvassed the precautionary principle elsewhere in this submission.

5. SOCIO-ECONOMIC CONSIDERATIONS, FOOD SECURITY, FOOD SOVEREIGNTY AND THE AGREEMENT ON THE APPLICATION OF SANITARY AND PHYTOSANITARY MEASURES (SPS AGREEMENT)

The provisions of the SPS Agreement creates certain obligations on South Africa which it must take into account in the course of the risk assessment, including the extent to which the approval of Monsanto's GM maize may negatively impact on the loss of production or sales in South Africa of locally produced non- GM maize.

Section 5(3) of the SPS Agreement provides as follows: " In assessing the risk to animal or plant life or health and determining the measure to **be applied for achieving the appropriate level of sanitary or phytosanitary protection from such risk,** Members shall take into account as relevant economic factors: the potential damage in terms of loss of production or sales in the event of the entry**, establishment or spread of a pest or disease; the costs of control or eradication in the territory of the importing**

Member; and the relative cost-effectiveness of alternative approaches to limiting risks."

We are particularly concerned about the detrimental impacts of cheap GM maize imports for animal feed in particular, on the production and sale of maize in South Africa. In this regard, we bring to the attention of the Executive Council that hundreds of thousands of metric tons of such GM maize are being imported into South Africa (especially from Argentina) by the animal feed industry because it is cheaper than if they were to purchase maize produced locally and thereby displacing and placing at risk thousand of jobs in the agricultural sector and related industries.

The Department of Trade and Industry (DTI) is represented on the Executive Council. It is our respectful submission that the DTI and the NDA should, as a matter of extreme urgency, conduct an assessment on the following:

- the socio and economic impacts of the importation of GM maize in the hundreds of thousands of metric tons by the animal feed industry in South Africa, from Argentina and the United States, including impacts on the production of maize in South Africa, the distortions in the market place caused by the sale of such maize and indeed, the long-term food security and food sovereignty impacts for South and Southern Africa, the predatory pricing policies of these grain exporters and the huge subsidy regimes available to them by their governments that assist in attaining those objectives of market domination and displacement of local producers.

We further refer the attention of the DTI, to several cases successfully undertaken by the government of Australia at the WTO regarding the permissible protection under the WTO rules, of its domestic grains/commodities market.

6. DEFICIENCIES IN THE GMO ACT REGARDING SPILLAGE OF GMOS DURING TRANSPORT

We are extremely concerned about the possibility that should Monsanto's application be granted, the provisions of section 2(2) of the Regulations to the GMO Act may be invoked and imports of GM maize into South Africa will take place without any biosafety oversight.

This concern is exacerbated by our profound disquiet concerning the negative environmental impacts that may arise from the spillage of whole GM maize grains during transportation and the milling process itself. We note with alarm that the transportation of GMOs as well as the mills to be used in the processing of GMOs is captured by the extraordinarily wide definition of contained use in section 1 the GMO Act. Contained use is defined to mean *"any activity in which organisms are genetically modified or in which such genetically modified organisms are cultured, stored, used, transported, destroyed or disposed of and for*

which physical barriers or a combination of physical barriers together with chemical or biological barriers or both are used to limit contact thereof, with the environment."

We strenuously dispute this definition, because the transportation of GMOs and indeed, the milling thereof, is in fact a release, requiring appropriate and adequate biosafety measures (which do not in any event exist in terms of the GMO Act) that are designed to prevent ecological harm. This is particularly pertinent given that the GMO Act exercises regulatory functions in respect only of those facilities where actual genetic modifications are conducted. Only academic and research institutions and bodies involved in genetic modifications under contained use, may be required to be registered.[4].

Our objections to the deeply flawed and biased provisions of the GMO Act cannot be overemphasised enough. We are aware that the government too, is cognisant of these regulatory deficiencies, and in this regard, Dr Julian Jaftha, the registrar of the GMO Act, has recently proposed five measures regarding the importation of genetically modified corn that have only commodity clearance in South Africa. According to the June 2004 issue of the Animal Feed Manufacturers' Association (AFMA) publication, the measures include the following:

- To address spillage or unintentional release during the importation of GM grain with only commodity clearance in South Africa, the transportation of imported whole GM grain is limited. Immediate milling of all consignments imported for use, as commodity in SA is necessary.
- Not all GM corn that have commodity clearance status (food and feed), have general release status as well. Thus, if only one event in the consignment does not have general release status, it means that the whole consignment is subject to immediate milling.
- Milling is to be done as close as possible to the port of entry to minimize the transportation of whole grain. The grain must be transported from the port of entry directly to the miller on a single trip without offloading and reloading until delivered at the miller.
- When applying for clearance, the importer must indicate where the grain is going to be milled and the mode of transport to be used. This information will help the Department of Agriculture to trace any spillage into the environment and to identify the responsible company.
- To prevent the purchase of GM material without informed consent, the seller of GM grains or grain products, e.g. animal feeds must clearly indicate the GM status of the consignment to buyers, as this may influence further trade negotiations and the use of these products.

(Source: Crop biotech update 23 July 2004)

These measures are welcome, but must, however, be given effect to in the Regulations under the GMO Act, for enforcement and compliance purposes. We also point out, that

we are not convinced that any or any proper monitoring has or will take place, to ensure that GMOs imported for food and feed does not cause harm to the environment as a result of spillage during import, transport and processing phases. In fact, we are not aware of any measures being taken by either the NDA or the Department of Environmental Affairs and Tourism of such monitoring.

SCIENTIFIC ASSESSMENT

APPLICATION BY MONSANTO SOUTH AFRICA

Monsanto Company represented by Monsanto South Africa has made application to the Department of Agriculture of South Africa "to enable the importation of grains for feed and food purposes that may contain maize grains derived from insect-protected maize line MON86 and maize hybrids MON863 X MON810"[5]. This includes the importation and use of the grains, but excludes cultivation.

NOTIFIER APPLICATION: AVAILABLE INFORMATION

The dossier supplied by Monsanto has several appendices designated as Confidential Business Information (CBI). These include:

Appendix 1

- Molecular analysis of MON863 maize
- PCR analysis and DNA sequence of the insert in corn rootworm event MON63
- Confirmation of the genomic DNA sequences flanking the 5' and 3' ends of the insert in corn rootworm event MON863

Appendix III

- Additional information concerning the 3' junction between the insert and the plant DNA- Maize line MON810
- Bioinformatics evaluation of DNA sequence from the 3' junction of the YieldGard® corn MON810 insertion event: assessment of predicted polypeptides
- Additional information concerning the 5' junction between the insert and the plant DNA – Maize line MON810

Appendix IV

- Safety assessment of Cry3Bb1 variants in corn rootworm protected corn

At the outset, it must be made clear that our ability to make a full independent scientific assessment is compromised by the large amount of data and information designated CBI. The lack of access to the full dossier of the notification compromises our ability to ensure that our concerns and interests will be taken into account.

In the following discussion, page numbers in brackets refer to the corresponding pages in the notifier application to the Department of Agriculture, South Africa.

BACKGROUND

Maize

Maize or corn (Zea mays L.) is grown commercially in over 100 countries primarily for the kernel, which is processed into a wide range of food and industrial goods[6]. The greater proportion of maize produced is used for animal feed with under 10% of the maize used as human food products. Starch produced from maize is converted into sweeteners, syrups and fermentation products[6].

Maize propagation is dependent on human intervention

Bacillus thuringiensis: Mode of Insecticidal Action

Bacillus thuringiensis (Bt), a common soil bacterium produces insecticidal proteins during sporulation. Each of the several thousand strains of Bt that exist produces its own unique insecticidal crystal protein (delta endotoxin)[7], each of which displays differing insecticidal activity, but with a similar mode of action. Typically, ingested delta endotoxins are dissolved in the insect midgut liberating the protoxins of which they are comprised. These undergo proteolysis and one of the fragments binds to the cells of the insect midgut epithelium, disrupting the osmotic balance and forming pores in the cell membrane causing cell lysis, gut paralysis and death within a few hours of ingestion[7,8].

THE GENETIC MODIFICATIONS AND MOLECULAR CHARACTERISATION

MON863

MON863 (designation MON-ØØ863-5) was produced to express the cry3Bb1 gene from Bacillus thuringiensis subspecies kumamotoensis to control infestation of Coleopteran species especially corn root worm (*Diabrotica* sp.)[9]. This gene was introduced by biolistic transformation into the publicly available inbred maize line A634[9]. Additionally, genetic modification includes transfer of the *nptII* gene from *Eschericihia coli* expressing the enzyme neomycin phosphotransferase II (NPTII) which confers resistance to particular aminoglycoside antibiotics9 such as kanamycin and neomycin.

Both introduced genetic elements are under the control of the CaMV 35S promoter with 4 repeats of an activating sequence in the case of the *cry3Bb1* gene. The resultant cry protein contains 653 amino acids and differs from the wild type by the addition of an alanine residue at position 2 and by seven amino acid changes. The neomycin phosphotransferase II encoding gene cassette includes the 3' untranslated termination sequence from the Agrobacterium tumefaciens nopaline synthase gene and an 153 base pair portion of the bleomycin (*ble*) binding protein gene. The total *nptII* cassette is 378 base pairs in size[9].

MON810

MON810 (designation MON-ØØ81Ø-6) marketed under the trade name YieldGard was developed by specific genetic modification to resist attack by the European corn borer[10] (*Ostrinia nubilalis*). The introduced genetic element from *Bacillus thuringiensis* subsp. *kurstaki* is under the control of an enhanced CaMV 35S promoter and maize heat shock protein (HSP70) intron. The terminator sequence was lost during integration and the resultant Cry1Ab insecticidal protein is truncated (91kD as compared to 131kD in the native form). Genetic modification was by particle acceleration (biolistic transformation) of maize line Hi-II with a mixture of plasmid DNAs[10].

MON863 X MON810

The MON863 X MON810 has been produced by the conventional maize breeding events through crossing the progeny of MON863 with those of MON810[11].

Comment on Molecular Characterisation

MON810 produces a truncated Cry protein. No detail is provided in the notifier application as to whether this difference in molecular weight between the transgenic line and the native form is significant or whether any evaluations have been conducted for environmental or food safety. Additionally, the loss of the terminator sequence raises the possibility that there might be an open reading frame comprising the genetic insert and flanking regions. The notifier does, not explain what the significance of the loss of the terminator sequence is.

MON863 contains a missing 10bp fragment from the end of PV-ZMIR13L, the significance of which is not explained by the notifier. There is also a *ble* gene after the stop codon of the *nptII* sequence, the presence of which creates an open reading frame.

Characterisation of the hybrid line is assumed to be the equivalent to the sum of the characterisations of the individual parent lines. The Food and Agriculture Organisation of the United Nations (FAO) have pointed out that the potential risks and benefits of genetically modified organisms (GMOs) need to be carefully assessed on a case-by-case basis[12]. It cannot be assumed that the two inserts act independently and that there are no interactions between the parent lines.

CaMV Promoter

The cauliflower mosaic virus (CaMV) is a DNA-containing para-retrovirus replicating by means of reverse transcription. It contains within its genome a viral promoter called 35S, a general strong plant promoter which has been used to secure expression of transgenes in a large proportion of commercialised GMOs. There are several studies indicating the potential for transcriptional activation of the 35S CaMV promoter in mammalian systems[31,13,14].

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 a very non-specific

manner[15]. 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[16], carcinogens and mutagens as well as to reactivate dormant viruses.

Detractors claim that virus infected cabbages and cauliflowers have been consumed for years with no ill effects and that similar pararetroviral sequences occur widely in plants, causing no apparent harm[17]. 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 the transformed plant where the natural regulatory mechanisms are not present[16]. 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[18].

GENETIC MODIFICATION: POSSIBLE UNINTENDED EFFECTS

Despite the expression of the introduced gene sequences having been confirmed by molecular characterisation and protein expression analysis, unintended effects that are not detected in the lab and that may only become apparent in the long term, cannot be ruled out. There are possible unintended effects of the presence of non-native fragments in the transgenic plants. The inserted gene sequences may interrupt native gene sequences and/or their promoters.

What is of concern here is the possible production of novel proteins from the transcription of unintended fragments. It cannot be assumed that unintended fragments are non-functional fragments or not transcribed and any such claim needs to be subjected to greater scrutiny and more investigation. Extra gene fragments in Monsanto's Roundup Ready Soya were also claimed to be non-functional and not-transcribed[19], but were later found to be transcribed to produce RNA[20,21,22].

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[23] necessitates more rigorous research into possible position effects prior to the granting of any release of transgenic organisms into the environment. Transformation by particle acceleration (biolistic) is associated with multiple fragments and gene rearrangements[24,23].

GENE EXPRESSION

Comment needs to be made on the trials from which the gene expression information has been generated. The field trial data is reported by Dudin et al 2000 and 2001[5] for a single season of testing of MON863. In order to obtain valid risk assessment data, several years expression levels obtained across a range of growing conditions according to protocols that allow meaningful statistical analysis are necessary[25]. Samples were

collected at only four sites in 1999 - two in Iowa and one each in Nebraska and Illinois. Additional pollen samples were collected from three sites in Argentina. It is not clear if pollen from U.S.-grown plants was used and if not, why not. Four replicated plots were grown at each of the four field trial locations. For mature roots, a single sample was collected at three of the four locations; two plants were sampled and served as a replicate, so in total expression levels in mature roots were sampled from just 6 plants. The pollen sources are even fewer. Only one composite sample from U.S. field trials was taken over a 7-day period at the Monmouth, Illinois site[25]. Twelve additional samples of pollen were tested from corn grown in Argentina. In grain, one replicate per site was sampled, involving a composite of 28 to 41 ears of corn. The rationale behind the sampling protocol and the use of composite samples is not clear. Nevertheless, in the absence of independently reported gene expression data, we have no option but to make an assessment of the notifier-reported results.

An assessment of the information provided by Monsanto regarding the expression levels of the novel protein Cry3Bb1 indicates higher expression in grain in the hybrid (61.1 μg/g fw) than in MON863 (42.7μg/g fw) (Page 61)[5]. Similarly, the levels of Cry1Ab were higher in the hybrid (0.84 μg/g fw) than in MON810 (0.46 μg/g fw) (Page 62)[5]. NPTII protein expression level in grain was relatively low in both parental and the hybrid lines, though expression was higher in young leaves and forage samples (Page 62)[5]. In grain, the level of Cry3Bb1 protein grain exceeds the level in roots almost three-fold at harvest time. Monsanto have calculated a margin of exposure (MOE) for these proteins using upper bound estimates of daily human maize consumption (Page 79). A MOE ≥ 100 (Page 80) is stated by Monsanto as being "generally regarded as being adequate to protect human health"[5]. These margins are based on consideration of toxins and not allergens[27], which can elicit a response at much lower levels. The application of this MOE to a determination of potential allergenicity is therefore spurious.

ALLERGENICITY OF ANY NEW PROTEINS

- *Assessment of Allergenicity*

The nature of genetic modification of higher plants results in the production of novel proteins which might cause allergic reactions. 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[46]. Cry1Ab, for example, has three characteristics of allergenic proteins, namely digestive stability, heat stability and structural similarity to vitellogenin, an egg yolk allergen[26].

It does not follow, from the observed lack of harmful effects by the notifier, of Cry3Bb and cry1Ab in commercially available insecticides, that the protein products of MON863 and the hybrid line are equally innocuous (Page 73). The expressed Cry3Bb1 protein in the transgenic lines differs in several important respects (7 additional amino acids) from

the wild type form as does the truncated Cry1Ab. There is no demonstration of a safe history of consumption of MON863, as stated by the notifier, as MON863 was only deregulated in the United States in 2002[27]. Also, foliar applied Bt sprays break down rapidly in sunlight and the chances of human exposure are minimal[26].

In instances where there has been exposure, e.g. on farms where farm workers were exposed to conventional Bt sprays, 2 out of 123 workers exhibited sensitivity to the Bt formulation even though it occurred at much lower concentrations than found in MON863 and the MON863XMON810 hybrid[28]. Aerial spraying of Bt pesticides precipitated increased respiratory health effects in local residents[29].

- *Allergenicity Tests*

Determinations of potential allergenicity were made by conducting *in vitro* digestion tests (Page 81) using simulated gastric fluid (SGF) and by comparing the transgene sequences to known allergen databases. The notifier states that a "correlation between digestibility in SGF and food safety has been previously validated". The EU Scientific Steering Committee notes that 'no absolute correlation exists" between pepsin degradation and allergenicity[27]. The Scientific Steering Committee makes further recommendations for determining allergenicity. This includes further testing such as serum binding, IgE binding, analysis of cross-reactivity and/or sensitising potential. Given that no findings on theses tests are reported, we can only assume that these tests were not conducted. The Cry3Bb1 protein was observed to degrade from a size of about 74kDa to fragments as small as 57kDa (Page 82). No evidence is provided that the breakdown product was characterised and evaluated with regard to the hazards linked to its biological activity as required by the guidelines of the EU Scientific Steering Committee[27].

Amino acid sequences of the MON863 Cry3Bb1 protein were compared to protein sequences within an allergen database (Page 76). As part of this comparison, an algorithm was developed to determine if the MON863 Cry3Bb1 protein shared a match of eight or more linearly contiguous amino acids (Page 76) to any sequence within the allergen and gliadin database. On page 82, however, the notifier states that the "hypothetical minimum requirement for a peptide to elicit an allergic response would be a six (to 15) amino acid linear epitope". It is not clear why the algorithm does not take this into account and searches instead for an eight amino acid sequence. This calls into question the Monsanto claim that there is no match to immunologically relevant amino acid sequences.

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[30],[31]. It is most commonly

recognized as infectious transfer[32]. 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[32]. 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.

- *Use of 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[31].

- *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 MON863 (*nptII*), there is a potential for gene transfer of these markers to pathogenic organisms. In MON863 the encoded product inactivates aminoglycoside antibiotics such as kanamycin and neomycin. Kanamycin, contrary to popular belief (page 105 of Monsanto application), is still used in medical applications, e.g. prior to endoscopy of the colon and rectum[33] and to treat ocular infections[34]. It is well known that there is cross resistance between antibiotics of a particular type[31]. Neomycin was found to cross react with kanamycin B in inhibiting RNAse P ribozyme 16s ribosomal RNA and tRNA maturation[35]. Other aminoglycoside antibiotics including streptomycin, gentamycin and tobramycin, which are used to treat human disease, have exhibited cross resistance[31]. 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.

- *Resistance of DNA to Digestion*

Monsanto argue that gene transfer is unlikely as the protein is rapidly degraded under conditions with simulate mammalian digestion. There are however several reported cases in the literature of both the persistence and transfer of gene sequences after ingestion of GM products. Polymerase chain reaction (PCR) has been used to demonstrate the presence of large fragments of M13 phage DNA, which had been fed to mice, in the faeces and bloodstream and in white blood cells[36]. Research published by the UK government in 2002 has shown that bacteria in human intestines had in fact taken up a novel gene from processed food containing GM Soya[37]. It has been reported that people with ileostomies (i.e. who make use of a colostomy bag) are capable of acquiring and harbouring DNA sequences from GM plants in the small intestine[38]. Recombinant DNA

fragments and Cry1Ab protein was also found in the gastrointestinal contents of pigs fed genetically modified corn[39].

- *Role of the CaMV Promoter*

The presence of the 35S CaMV promoter, which is known to be active in micro-organisms,[40] would facilitate the transfer of the antibiotic resistance marker from the plant products of the *nptII* gene to bacteria in the intestines of humans and livestock.

- *Main Findings Regarding HGT and theARGMs*

The main findings regarding horizontal gene transfer and the presence of selective antibiotic markers therefore are:

that there is evidence of gene transfer from plant DNA to bacteria in the human gut;

the *nptII* gene is under the control of a promoter that can be utilised by intestinal bacteria, and

the claim of DNA breakdown by the notifier is contradicted by the published research.

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)[41]

FEEDING STUDIES

The notification provides summary findings of the feeding studies without detailed reports and results of the studies. The studies have been conducted by Monsanto and have not been peer reviewed and subjected to independent scrutiny. The lack of detailed information makes an assessment of the validity of the protocols and analysis of the data almost impossible. The feeding studies referred to in the Monsanto application do not make clear whether the naturally occurring protein (surrogate) or GM plant-produced protein is used as feed. Given the prevalence of the former being used by developers of biotech plants, we will assume that this is the case here.

In the United States, animal testing of genetically modified plants for acute toxicity is limited to a 28-day rodent feeding trial that employs a surrogate bacteria-generated version of the plant-produced GM protein[46]. This may differ in several important respects from the GM protein. Further questions to be asked of feeding studies, such as long term impacts to detect chronic or reproductive effects, cannot be answered by such

short-term feeding studies. Occasionally, longer term feeding studies are carried out, but the results not always reported.

Monsanto Corporation conducted a 90 day sub-chronic toxicity rat feeding trial with MON863. The French commission on Genetic Engineering (*La commission du genie biomoleculaire francaise*) reviewed this study and noted a number of significant differences between the control group, fed conventional corn, and the rats fed MON863. Of the rats fed MON863, males exhibited higher lyphocyte levels and more kidney anomalies and females reduced levels of reticulocytes (immature red blood vessels) and significantly increased blood sugar levels[42]. Monsanto Corporation has refused to hand over to the German government the detail of this study on the grounds that the information was CBI[46]. All this achieves is to cast doubt on the claims by Monsanto regarding the validity of their feeding studies.

ASSESSMENT OF THE HYBRID

MON863 X MON810 is a hybrid of two lines, viz., MON863 and MON810, both of which have reported irregularities in their molecular structure[5]. MON863 for example contains a transcribed open reading frame and MON 810 produces a truncated protein due to the loss during recombination of NOS termination codon of the genetic insert. This raises several questions regarding the genetic characterisation of the hybrid. The information provided by the notifier relates to the parental lines and not the hybrid form, with the notifier claiming "negligible likelihood for any significant molecular interactions between the inherited modification events, when they are present in combination in MON863 X MON810". The basis of such a claim is not clear and the notifier does not substantiate. It cannot be assumed that the genetic inserts act independently or that no interaction has taken place between the parental lines. It is also not clear if transgene stability has been established for the hybrid[43].

The Food Standards Agency which is the Competent Authority for assessment of novel foods in the United Kingdom, on 4 August 2003, in terms of EC guidelines, presented a reasoned objection to the Initial Opinion by Germany regarding the placing on the market of grains and grain derived food ingredients from both MON 863 and MON863 X MON810[44]. This was done on the grounds that the Advisory Committee for the Assessment of Novel Foods and Processes considered the available safety information for the hybrid line insufficient. The data presented on the hybrid line was considered limited and the approach of making a safety assessment by reference to data of the individual parent lines rather than the hybrid, inadequate. The reduction in mineral content in the compositional data supplied by the notifier was considered to be an area that warranted further investigation[44].

ENVIRONMENTAL CONCERNS

Dissemination and accidental release of the GMO is considered "highly unlikely" (risk assessment), but the potential does exist however, as acknowledged by Monsanto in their application (Page 116), of "wider release of imported grains within South Africa e.g., through misuse"[5]. Experience of similar imports of maize for food and feed without cultivation in Mexico contradicts the Monsanto claim of "negligible risk"[5]. Local maize landraces were found to be contaminated with GMO constructs[27,45]. This was thought to be as a result of the inadvertent planting of GM maize grains that had been sold for food and feed. There is no assurance that this will not happen in South Africa and a monitoring plan is therefore essential to ensure that maize grains sold for food and feed are restricted to this purpose.

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[46]. 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[46].

Uncertainty is a key element of the Biosafety Protocol (Cartagena Protocol on Biosafety to the Convention on Biological Diversity[47]). 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 use as a reason for postponing cost-effective measures to prevent environmental degradation". The discussions above have identified potentially dangerous effects from the use of MON863 and the hybrid MON863 X MON810. Further the available scientific information, as provided by the notifier, does not allow for a full evaluation or determination of the associated risks of the use of the said transgenic lines.

COMMENT ON THE NOTIFIER APPLICATION RESPONSES

The response to question 7 (page 19) is addressed as if the maize plant and the genetically modified line are one and the same. For reasons, outlined elsewhere in this document we do not believe this to be an adequate response.

The over 40-year history of safe use of Bt based sprays (page 72) as widely used by organic farmers does not necessarily extend to the genetically modified plant. It cannot

be assumed that the naturally existing insecticidal protein is equivalent to the genetically modified form. The bacteria generated protein may differ in several important respects from the GM-plant protein and it does as detailed in the description of the different transgenic lines above. It is common for developers of GM plants to carry out supporting studies using the naturally occurring or surrogate proteins, rather than the GM plant-produced product. This is usually because it is time consuming and expensive to isolate adequate quantities of transgenic proteins from transgenic crop lines. The practice of using surrogate proteins has been widely criticised in particular by expert committees of the National Academy of Sciences[48] and the Environmental Protection Agency[49,46].

The designation of several appendices as CBI does not allow for an informed critical assessment of the intended introduction of the product onto the market. The available evidence is insufficient for a meaningful evaluation of the associated health and safety risks. Failure to obtain access to the PCR data has precluded us form making any determination of the effectiveness of the PCR or whether any further testing is necessary.

The notifier makes the claim that the genetic modification does not introduce any new category of risk as compared to risks from conventional breeding. This is not to be taken as an apparent truth. 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[46].

In consideration of the safety of hybrids, safety assessments need to be carried out on the hybrid lines, rather than on individual parent lines, so that interactions between the two sets of inserted genes can be assessed.

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