Objection to Monsanto's application for an extension permit for field trials of Mon 87460

Prepared for the African Centre for Biosafety by Dr. Shenaz Moola

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INTRODUCTION

In 2007 Monsanto South Africa applied for and was granted a trial release permit to conduct field trials with maize event MON87460. This was a multi-season permit allowing for the field trials to continue for three seasons commencing 2007/2008. Approval for field trials on Mon 87460 in Delareyville, Lutzville and Pretoria were granted in 2009 to Monsanto and an extension for the field trials granted in 2010. The current application before the South African Department of Agriculture, Forestry and Fisheries (DAFF) is for a further extension to these field trials on the grounds that the time previously applied for and granted is inadequate in the face of unpredictable biotic (insect damage) and abiotic factors (excessive rains, drought)

The field trials of MON87460 by Monsanto are presented as forming part of the larger Water Efficient Maize for Africa (WEMA) Initiative. WEMA is a public-private initiative led by the African Agricultural Technology Foundation (AATF) and involves a partnership between the national agricultural agencies from Kenya, Uganda, Tanzania, Mozambique and South Africa, the Maize and Wheat Improvement Center (CIMMYT) and Monsanto. This forms part of the AATF's Drought Tolerant Maize for Africa (DTMA) Project.[1]

The South African regulatory framework for public input and comment, regarding the introduction of Living Modified Organisms (LMOs) into the environment, is extremely limiting and does not allow for meaningful engagement by civil society regarding these applications. As a result, the African Centre for Biosafety (ACB) was unable to submit comment on the initial 2007 Monsanto applications within the constraints of the timeframe imposed by the regulations. However, in May 2007, the (ACB) placed on record its concerns about the granting of the field trial permits (17/3(4/07/015) and 17/3(4/09/242)). Further, the ACB submitted an objection to the 2010 extension application reiterating our initial concerns and placing on record our objection to the continued exposure of the environment to genetically modified maize and our objection to the granting of these applications.

These comments are in respect of an application (non-CBI) by Monsanto for a further extension submitted to DAFF in May 2011.

STATUS OF APPROVAL OF MONSANTO'S DROUGHT-TOLERANT MAIZE IN THE USA

It was reported in May of this year (2011) that the United States Department of Agriculture looks set to grant a Monsanto petition for the unlimited sale of maize variety Mon 87460 genetically engineered by Monsanto Co. to resist drought.[2] In North America alone, up to 40 percent of crop-loss insurance claims are due to heavy

or moderate drought with the worldwide crop loss average as high as 15 percent of the annual crop.[3] The USDA conducted a draft environmental assessment, currently out for public comment,[4] but also noted that many maize varieties on the market match Monsanto's strain in their water use. Specifically, it was stated in the assessment that:

“When grown in water-limited field and greenhouse conditions, MON 87460 corn exhibits classic drought sensitivity symptoms, including reductions in yield, plant height, ear height, seedling vigor, and expected changes in plant height, chlorophyll content, and leaf roll” [5]

Furthermore, it was stated that

“Taken in total, these data demonstrate that the negative effects of drought stress in MON 87460 are not alleviated and strongly suggest that areas unable to support economically viable production of conventional corn will also not support production of MON 87460”[5]

and that

"Equally comparable varieties produced through conventional breeding techniques are readily available in irrigated corn production regions."[5]

OUR MAIN CONCERNS

We have previously raised several concerns about the event in question. These relate to uncertainty regarding the nature of the modification, how realistic the anticipated developer outcome is, possible adverse ecological impacts of introduction of the event into the environment and a request for consideration of more readily available, more easily implementable, less costly and more sustainable alternatives.

THE NATURE OF DROUGHT AND DROUGHT TOLERANCE

Water plays a crucial role in the survival of plants by fulfilling the roles of solvent, transport medium and evaporative coolant as well as providing the energy necessary to drive photosynthesis, the natural plant process which synthesizes organic food.[6] Under conditions of drought, water loss in plants may result in negatively impacting plant metabolism. Water deficiency is a severe limiting factor in several countries and impacts on both food production and the economies of these countries. Approximately four tenths’[7] of the world’s agricultural land is in arid or semi-arid regions with transient droughts causing death of livestock, famine and social

dislocation. Several agricultural regions are reliant on irrigation to maintain yields. Those crop plants which can make the most efficient use of water and maintain acceptable yields will be at an advantage in these regions.

Research into drought tolerance and mechanisms for improving drought resistance are underway internationally to provide solutions to the problems of water deficiency, to save water used in agriculture and to ensure the development of sustainable agriculture. This includes research into elucidating the mechanism of drought tolerance in plants – different plants have different genetic makeup and hence different abilities for drought tolerance.

Drought tolerance is an extremely complex phenomenon mediated by multiple genes and regulatory pathways and from the reported literature, has been shown not to be as easy to engineer into plants as more simply inherited traits governed by single genes. The coding for drought tolerance, is incredibly complex with up to as many as 60 genes implicated, all interacting in a subtle and complex way. The successful manipulation and transfer of many complex genes, which can respond to a variety of conditions, and not produce unwanted toxins and allergens, is a long way off for current scientific knowledge with some geneticists admitting that even hoping for drought tolerance in the next 10 or 20 years may be too ambitious.[8]

In the Monsanto Summary that forms part of the application for placing of Mon87460 on the EU market, there is an admission by Monsanto that under very dry conditions, precisely the conditions under which WEMA is attempting to develop new crop varieties, the drought tolerant trait may not be effective in producing a viable yield: [9]

"Under well-watered conditions, grain yield for MON 87460 is equivalent to conventional maize. Under water-limited conditions, grain yield loss is reduced compared to conventional maize. However, like conventional maize, MON 87460 is still subject to yield loss under water-limited conditions, particularly during flowering and grainfill periods when maize yield potential is most sensitive to stress, by disrupting kernel development. Under severe water deficit, maize grain yield for MON 87460, as well as conventional maize, can be reduced to zero."

What then is the benefit of such extensive research and development effort? On the other hand, if, as Monsanto claims, the maize does confer some advantage under water-stressed conditions, then the potential for proliferation and persistence must be re-assessed within this context and cannot be considered to be limited to those areas with a lower limit of summer rainfall of 15cm.[10]

GENETIC MODIFICATION

NATURE OF THE MODIFICATION

In order for the ACB to submit a full and comprehensive response to DAFF, it needs to have a complete view of the molecular characterisation on MON 87460 so as to understand the genetic material introduced into the host genome. At a minimum, this includes:

1. Details on the transformation method together with a detailed description of the introduced DNA sequences;

2. The characterisation of the inserted DNA including any rearrangements that might have occurred during transformation, and

3. Information of the genetic stability of the inserted DNA and any accompanying expressed traits.

4. Extensive sequence data on both the constructs and flanking sequences in the final hybrid and in subsequent generations of offspring

From the Monsanto Application to DAFF we have been supplied with limited molecular characterisation information, detailed below.

MON 87460 was developed through Agrobacterium-mediated transformation of conventional maize variety embryos and expresses cold shock protein B (CspB) from Bacillus subtilis and nptII from Tn5 of Escherichia coli. The *nptII* gene is a marker gene conferring resistance to antibiotics (as kanamycin and neomycin) on the plant and the *CspB* gene encodes cold shock protein B (CSPB) intended to limit yield loss under water-limited conditions compared to conventional maize.

The MON 87460 event includes the nptII expression cassette flanked by two functional loxP sites. The loxP recombination site is recognized by the P1 bacteriophage Cre recombinase. Given that: 1) the *nptII* gene is regulated by the 35S promoter that is known to be active also in bacteria, and 2) the functional antibiotic resistance marker is flanked on both sites by loxP recombination sites, an enhanced recombination potential in bacterial environments with functional CRE proteins is present.

Thus, it is reasonable to assume that the *nptII* gene in this event has an increased likelihood of successful recombination and expression in exposed bacterial recipients. The Cre producing PI bacteriophage is known to have a broad host range and can be found in a range of bacteria naturally present in the gastrointestinal tract of mammals and humans.[11]

The information of what Monsanto has identified as the functional elements are detailed in two of the applications (pages 2 and 3) with “any section of the vector not listed in Table 1 but shown in the plasmid map” being “non-transcribed vector sequences that do not contain any functional genetic elements”. No further detail is provided on these or on the open reading frames present as a result of the modification.

The lack of complete sequence information makes an assessment of the gene expression cassette nigh impossible. Agrobacterium-mediated transformation is characterized by multiple fragments and gene rearrangements.[12,13] 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,[14] but were later found to be transcribed to produce RNA.[15,16.] Unintended effects that are not detected in the lab and that may only become apparent in the long term cannot be ruled out.

Monsanto conducted theoretical bioinformatic analysis to determine what theoretical proteins may be for coded for in open-reading frames.[17] No detail of the particular approach is provided and it is not clear whether the model applied would enable predictions of unintended expression, regulatory and pleiotropic effects.

Furthermore, the applicant does not give any information of the functional consequences on the expression or regulation of the recombinant protein or for endogenous gene expression, or even further bioequivalence studies to substantiate the claim of equivalence of bacterial versions of the transgenic protein used in safety testing and the actual (admittedly modified from the native) protein produced by the host maize plant.[11]

HORIZONTAL GENE TRANSFER (HGT) AND ANTIBIOTIC RESISTANCE MARKER GENES

An antibiotic resistance marker gene (ARMG) has been used in the development of MON87460. Specifically, the *nptII* gene from *Escherichia coli* which expresses the enzyme neomycin phosphotransferase II (NPTII) has been used. NPTII inactivates principally kanamycin, geneticin and neomycin by phosphorylation.

Horizontal gene transfer (HGT) is the transfer of genetic material between organisms, outside the context of parent to offspring reproduction[18],[19] typically by 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.

Kanamycin, contrary to popular belief, is still used in medical applications, e.g. prior to endoscopy of the colon and rectum[21] and to treat ocular infections[22]. It is well known that there is cross resistance between antibiotics of a particular type[19]. Neomycin was found to cross react with kanamycin B in inhibiting RNAse P ribozyme 16s ribosomal RNA and tRNA maturation[23]. Other aminoglycoside antibiotics including streptomycin, gentamycin and tobramycin, which are used to treat human disease, have exhibited cross resistance[19]. 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.

In the development of MON 87460, the residual *nptII* gene is gratuitous especially since it is bordered by loxP sites and thus could have been removed. Since the gene and trait are unnecessary and could be removed, why was this not done by the developer to minimise the potential risks through HGT?

Monsanto cites the European Food Safety Authority (EFSA) Statement [24] that antibiotic resistance markers have "no adverse effects on human health and the environment" in support of its use of an ARMG (C-page1). The EFSA opinion is not as unequivocal as is suggested by Monsanto. What EFSA does says, is"

"Kanamycin and neomycin are both categorized by the WHO Expert Group on Critically Important Antimicrobials for Human Health as 'Highly Important Antimicrobial'. Kanamycin is used as a second-line drug for the treatment of infections with multiple drug-resistant tuberculosis (MTB). The increasing occurrence worldwide of "extensively drug-resistant" (XTB) isolates of MTB with resistance to second-line antibiotics such as kanamycin is a cause for global concern."[24]

and;

"There are limitations related among others to sampling, detection, challenges in estimating exposure levels and the inability to assign transferable resistance genes to a defined source. ***The importance of taking these and other uncertainties described in this Opinion into account requires to be stressed****."*[a,24]

"Notwithstanding these uncertainties, the current state of knowledge indicates that adverse effects on human health and the environment resulting from the transfer of these two antibiotic resistance genes from GM plants to bacteria, associated with use of GM plants, are ***unlikely"****.*[a]

[a] Own emphasis

Further, two senior from EFSA's biohazard panel (Dr. Christophe Nguyen-Thé and Dr. Ivar Vågsholm), which was jointly responsible for the assessment, did not agree with the conclusions of the EFSA statement. They countered that "adverse effects […] cannot be assessed" and that the probability of gene transfers from plants to bacteria ranges widely "from unlikely to high". Their objections are included in the EFSA statement.[24]

Monsanto responded to the above ACB comments by stating that ""The Chairs responded by confirming that the scientific issues related to the minority opinions have already been extensively considered during the preparation of the joint scientific opinion and the formulation of the conclusions therein and thus, from a scientific perspective, further clarification of the joint scientific opinion is not required, nor is further scientific work needed at this time…". Monsanto therefore considers the EFSA statement and the conclusions reached therein to be trustworthy, and will cite it in context where relevant."[17]

The ACB asserts Monsanto's the right to quote its selected sources. The above discussion by ACB is intended to illustrate that the debates around the use of ARMGs are not as cut and dried as is suggested in the Monsanto application and that there are alternative positions that cannot simply be dismissed out of hand and much work needs to be done before conclusive statements can be made on the use of ARMGs.

The concern that ARMGs that are present in genetically modified organisms may inactivate antibiotics, which are in clinical or veterinary use, is habitually dismissed on the basis that resistance to such antibiotics is already common in soil bacteria. In respect of safety assessments however, this view ignores the fact that the kinds of bacteria that cause disease in people and animals are not soil bacteria and the resistance is not, or previously has not been, common in these pathogens.[25,26]

In Europe there is extensive argument that the EFSA GMO Panel's opinion does not necessarily reflect the more precautionary motivated regulations of ARM genes for commercial use in food and feed in Austria, or in Hungary for example.[27]
Furthermore, there is concern that in making the statements that it has, EFSA has effectively EFSA has over-written the EC regulation and overruled its prior opinionb from 2004 without any new data on the probability of horizontal gene transfer.

RESISTANCE OF DNA TO DIGESTION

There are several reported cases in the literature of both the persistence and transfer of gene sequences after ingestion of GM products. Polymerase chain

[b] Directive 2001/18/EC, which requires a step-by-step phasing out of ARM genes in GMOs, which may have adverse effects on human health and the environment (Art. 4 (2) Dir. 2001/18/EC) by the end of 2004

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[28]. 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[29]. 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[30]. Recombinant DNA fragments and Cry1Ab protein was also found in the gastrointestinal contents of pigs fed genetically modified corn[31].

No detail is given on the Stability and digestibility of CSPB or CSPB:nucleic acid complexes, if any, to enable the ACB to comment.

POLLINATION

VOLUNTEERS

The Monsanto application to the South African government states that current agronomic processes will control any maize volunteers (page 8). It is not expected that the GE maize will become a persistent or invasive weed, should a seed spill or inadvertent planting occur; however, maize plants have been shown to survive over a growing season, under comparatively colder conditions[32] than found in South Africa. The difficulty with genetically modified plants is that they cannot be distinguished from conventional maize by visual inspection – one maize plant looks much like the other, genetically modified or not. As a result, volunteers may go undetected. Should any volunteers arise, the resulting pollen could cross-pollinate with maize in adjacent fields, producing genetic contamination.

POLLINATION DISTANCES

"Monsanto is committed to effectively isolate the trials from any conventional maize growing in the environment surrounding the trial site" (page 6)

We know that: 1) Maize is an outbreeding species that produces very large amounts of pollen and 2) Measurement of pollination distances for maize follow a leptokurtic distribution pattern, i.e., cross-pollination rapidly declines as the distance from the donor field increases.[33] A very comprehensive study on cross-pollination of maize has shown that:

1. Cross-pollination between two fields of maize at 200m occurs at levels greater than 0.1%;
2. For one of the three years in the study, cross-pollination of 2.47% was recorded at 200m from the source; and

3. a three-year mean of 1.19% cross pollination, over 11 times more than 0.1%, suggests that cross-pollination above 0.1% is a typical rather than an exceptional occurrence.[34,35]

Recently conducted research by the University of Exeter applied a new method for predicting the potential for cross-pollination, which takes account of wind speed and direction. The findings showed huge variation in the degree of cross-pollination between GM and non-GM crops of maize, oilseed rape, rice and sugar beet.[36] The levels vary depending on whether the GM field is upwind or downwind of the non-GM field. Current guidelines relating to field-to-field distances do not take into account this variation. If the GM field in a trial is downwind of the non-GM field, the trial will underestimate the potential for cross-pollination.[36]

The likelihood that even the strictest isolation distances will completely eliminate cross-pollination is very low. Studies on canola pollen flow have found seeds up to 3km away even though most falls within 100m from the source.[37] Wind currents can also hinder the effectiveness of this strategy with pollen transported to high altitudes by wind and being deposited on fields long distances away from the source without being challenged by the height of pollen barriers (such as trees).[38,39]

Seed flow is a more complicated issue because seeds can have a dormancy of years, allowing their continual movement by vectors such as animals and human activity. Contaminated harvesting equipment and transport vehicles quickly transgress spatial barriers, undermining the ability of most landscapes to harbor effective zones.[39]

In order to ensure isolation of new traits, robust measures have to be applied to prevent pollen mediated gene flow. Monsanto has not supplied any details of isolation distances or the proposed measures that it is “committed to” for effective isolation. There is no detail in the Monsanto application of the period for which fields will be monitored for volunteer growth and whose responsibility this is. Monsanto claim that pollen remain viable for about two hours[17] after atmospheric exposure. Other studies cite survival periods of up to 24 hours with cool temperatures and high humidity favouring longevity.[40]

ALTERNATIVES TO GE DROUGHT TOLERANT PLANTS

Several marker-assisted selection (MAS) techniques have been developed for the improvement of polygenic traits. The advances in the development of molecular tools has allowed for improved identification, mapping and isolation of genes in a wide range of crop species.[41] Initially, markers called restriction fragment length polymorphisms (RFLPs), were used to construct linkage maps for several crop species, including maize, tomato, and rice. Later the polymerase chain reaction (PCR)

revolutionized molecular marker assays because of the easy and suitable for automation.41 MAS needs to be complemented by traditional breeding programs especially in the case of drought where yield is regulated by several genes. Yet it remains a promising technique worthy of further investigation.

Traditional breeding methods and conventional selection have served farmers well in identifying drought tolerant plants. It is well documented that approaches to improving crop quality by enhancing soil quality greatly improves water retention, and generally improves crop growth, at much less cost. The US Rodale institute has carried out long-term comparisons between organic and conventional crops and found that during the drought years the organic yielded better because the soil holds more water.

The International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD),[42] an intergovernmental report modelled after the Intergovernmental Panel on Climate and commissioned by the World Bank was carried out over 4 years and involved the collaborative effort of more than 400 scientists. Adopted by fifty-eight countries in the global North and South (excluding the United States, Canada or Australia), the IAASTD found that a agro-ecological approaches to farming, focussing on small-scale sustainable agriculture, locally adapted seed and ecological farming better address the complexities of climate change, hunger, poverty and productive demands on agriculture in the developing world.[43] The interaction with scientists is essential in assisting farmers to improve conservation technologies and developing breeding strategies in a way that does not place additional burdens under communities in already straitened circumstances.

CONCLUSIONS

From our overview of the scant details provided in the non-CBI version fo the Monsanto application to DAFF, the ACB objects we conclude that:

1. The possibility of any real yield benefit to be derived from the transformed plants is not rated very high by Monsanto. The risks of exposing the environment the public and environment to such a product cannot be justified within this context.

2. The application is silent on the measure/s to be taken to prevent pollen flow and makes an assessment of the growing conditions impossible.

3. Incomplete molecular characterisation information and detail on subsequent genetic evidence to confirm the original transformations makes complete assessment of the transformation event impossible.

4. The development of the MON87460 event has not been optimised to minimise gene flow of ARMG and it is not clear why this was not done.

5. No health and safety and human health impacts from possible consumption of MON 87460, in the event of gene flow and/or handling spills, are included in the application. This hampers the public's ability to contribute or engage meaningfully in any discussions regarding GE foods or be able to make informed choices about matters that so closely impact on them. Monsanto state that "CspB has a history of safe consumption and nptII is present in several biotechnology-derived crops that have undergone previous safety assessments."17. There is no reporting from Monsanto however on compositional and nutritional equivalence of grain and forage from MON 87460 and conventional maize, or on any analyses that might have been performed specifically to establish the types of changes that might be unwanted (e.g. toxicological or immuno-stimulatory) or undesirable in exposed organisms.

6. More sustainable agro-ecological approaches to farming should be supported and promoted by DAFF. Such approaches help maintain soil diversity through crop rotations that balance soil nutrients and promote the use of natural readily available inputs like compost and manure which replenish the soil.

7. The consultation process is not sufficiently long to enough to enable full and meaningful public participation and the information made available to the public is kept to a minimum.

8. The original decision by the South African regulatory authority to permit field trials of MON87460 has not been made publicly available through the Biosafety as Clearing House (BCH) in terms of Article 20 of the Cartagena Protocol and constitutes non-compliance with the Cartagena Protocol, to which South Africa became a party in August 2003. 19 decisions regarding LMOs have been posted while the South African government has granted over 2000 permits since 1999. The ACB cannot therefore meaningfully respond to the original regulatory authority assessment. This also calls to question whether the resources and capacity within the South African DAFF are optimally geared to ensure thorough and complete assessment of applications for the introduction of GMOs into the environment.

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.

REFERENCES

[1] The Drought Tolerant Maize for Africa Initiative. http://dtma.cimmyt.org/ (accessed 05.04.2010)

[2] Monsanto. Petition for the Determination of Non-regulated Status for Mon 87460. Monsnato Petition No. 07-CR-191U. http://www.aphis.usda.gov/brs/aphisdocs/09_05501p.pdf (accessed 21.06.2011)

[3] NY Times. USDA Looks to Approve Monsanto's Drought-Tolerant Corn. http://www.nytimes.com/gwire/2011/05/11/11greenwire-usda-looks-to-approve-monsantos-drought-tolera-84634.html (accessed 18.06.2011)

[4] Department of Agriculture. Animal and Plant Health Inspection Service. [Docket No. APHIS–2011–0023]. Notice: Plant Pest Risk Assessment, and Environmental Assessment for Determination of Nonregulated Status for Corn Genetically Engineered for Drought Tolerance. http://www.gpo.gov/fdsys/pkg/FR-2011-05-11/pdf/2011-11485.pdf (accessed 22.06.2011)

[5] Monsanto Company Petition (07-CR-191U) for Determination of Non-regulated Status of Event MON 87460. OECD Unique Identifier: MON 87460-4. Draft Environmental Assessment. March 2011. http://www.aphis.usda.gov/brs/aphisdocs/09_05501p_dea.pdf (accessed 20.06.2011)

[6] Peters, S. What are the experts saying? Resurrecting hope: drought tolerant crops. Public Understanding of Biotechnology. http://www.pub.ac.za/issues/drought.htm (accessed 13.05.2007)

[7] Effects of Abiotic Stress on Plants. http://www.liv.ac.uk/~sd21/stress/drought.htm (09.04.2010)

[8] Anderson, T. (2006) UN Climate Conference - An Opportunity for GM?. Cambio climático y transgénicos.Proyecto de Bioseguridad Puerto Rico http://bioseguridad.blogspot.com/2006/10/cambio-climtico-y-transgnicos.html (accessed 03.04.2010)

[9] Application for authorization to place on the market MON 87460 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed. Part II. Summary.

(EFSA-GMO-NL-2009-70) (EFSA-Q-2009-00661) http://www.gmo-compass.org/pdf/regulation/maize/MON87460_maize_application.pdf (accessed 05.04.2010)

[10] Monsanto applications to the South African Department of Agriculture, Forestry and Fisheries (March 2010) for permit licenses and requests to extend field trials. Part II. Risk Assessment Responses. Point 18 (responses uniform across all 4 applications)

[11] Myhr, A.I. & Quist, D. Genok Centre for Biosafety. Impact assessment of maize MON 87460 from Monsanto (EFSA/GMO/NL/2009/70). 24.03.2010.

[12] Greenpeace comments on: SNIF for the deliberate release and placing on the EU market of the 1507 maize, C/ES/01/01. http://www.greenpeace.se/files/200-2399/file_2308.pdf (15.05.2007)

[13] Snow, G. A., Andow, D. A., Gepts, P., Hallerman, E. M., Power, A., Tiedje, J. M., and Wolfenberger, L. L. (2004) Genetically engineered organisms and the environment: Current status and recommendations. *Ecological Society of America Position Paper*. ESA Public Affairs

Office. February 26, 2004. http://ww.esa.org/pao/esaPositions/Papers/geo_position.htm (18.05.2007)

[14] Monsanto (2000) *Dossier containing molecular analysis of Roundup Ready Soya.* http://www.foodstandards.gov.uk/pdf_files/acnfp/dossier.pdf available at http://www.foodstandards.gov.uk/committees/acnfp/acnfpassessments.htm (accessed 18.05.2007)

[15] Monsanto (2002a) Transcript analysis of the sequence flanking the 3' end of the functional insert in Roundup Ready Soybean event 40-3-2. http://www.food.gov.uk/science/ouradvisors/novelfood/assess/assess-uk/60500 (accessed 18.05.2007)

[16] Monsanto (2002b) Additional characterisation and safety assessment of the DNA sequence flanking the 3' end of the functional insert of Roundup Ready Soybean event 40-3-2. http://www.food.gov.uk/science/ouradvisors/novelfood/assess/assess-uk/60500 (accessed 18.05.2007)

[17] Monsanto's response to objections from the African Centre for Biosafety to Monsanto's application for field trials of drought tolerant GM maize MON87460

[18] Heinemann, J. A. (2003) *Bioscience*. 12, 51 cited in [19].

[19] European Communities: Measures Affecting the Approval and Marketing of Biotech Products (DS291, DS292, DS293). (2004) Third Party Submission by Norway.

[20] Heinemann, J. A. Gene Ecology Guide to: Measuring Horizontal Gene Transfer. Condensed version of paper published in Nature Biotechnology in September 2004. *Personal Communication*.

[21] Ishikawa, H. Akedo, I., Minami, T., Shinomura, Y., Tojo, H. & Otani, T. (1999) Prevention of infectious complications subsequent to endoscopic treatment of the colon and rectum. *Journal Infect. Chemother.* 5, 86.

[22] Hehl, E. M., Beck, R., Luthard, K., Guthoff, R. & Drewelow, B. (1999) Improved oenetration of aminoglycosides and fluorozuinolones into the aqueous humour of patients by means of Acuvue contact lenses. *European Journal of Pharmacology.* 55(4), 317.

[23] Mikkelsen, N. E., Brannvall, M., Virtanen, A. & Kirsebom, L. A.l (1999) Inhibition of RNase P RNA cleavage by aminoglycosides. National Academy of Sciences, USA. 96, 6155.

[24] EFSA Statement. Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants" http://www.efsa.europa.eu/de/scdocs/doc/1108.pdf (accessed 08.04.2010)

[25] Heinemann, J. A., Ankenbauer, R. G. and Amábile-Cuevas, C. F. (2000). Do antibiotics maintain antibiotic resistance? Drug Discov. Today 5, 195-204.

[26] Heinemann, J. A. (1999). How antibiotics cause antibiotic resistance. Drug Discov. Today 4, 72-79.

[27] Scientific Opinion in Support of Hungary's Moratorium on GM Potato. http://www.biosafety-info.net/article.php?aid=702 (accessed 19.06.2011)

[28] Schubbert, R., Lettmann, C. Doerfle, W. (1994) Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. *Mol. Gen Genet*. 242, 495.

[29] Netherwood, T., Matin-Orue, S. M., O'Donnell, A. G., Gockling, S., Gilbert, H. J. & Mathers, J. C. (2002) Transgenes in genetically modified Soya survive passage through the human small bowel but are completely degraded in the colon. UK Food Standards Agency. *Research Report G01008: Evaluating the risks associated with using GMO in human foods.*

[30] Heritage, J. (2004) The fate of transgenes in the human gut. *Nature Biotechnology.* 22(2), 170. http://www.nature.com/cgi-taf/DynaPage.taf?

[31] Chowdhury, E. H., Kuribara, H., Hino, A., Sultana, P. and Mikami, O. (2003) Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn. *Journal of Animal Science.* 81, 2546.

[32] Crawley, M.J., Brown, S.L., Hails, R.S., Kohn, D.D. & Rees, M. (2001) Transgenic crops in natural habitats. *Nature*, 409: 682-683.

[33] Messeguer, M. P. J, & *Melé*, E. (August 2006) Gene Flow From GE to Conventional Maize Using Real-Time Pcr. http://www.isb.vt.edu/articles/aug0602.htm (accessed 09.04.2010)

[34] Jones, M.D., & J.S. Brooks (1950) Effectiveness of distance and border rows in preventing outcrossing in corn. Tech. Bull. No. T-38. Oklahoma Agric. Exp. Stn., Stillwater, OK

[35] An assessment of outcrossing in maize, relevant to the proposed decision to add GM maize variety Chardon LL to the National List Proof of Evidence of Professor Jean Professor Jean Emberlin BSc (Hons), PhD (Director) Director, National Pollen Research Unit University College Worcester on behalf of Friends of the Earth. October 2000. http://www.foe.co.uk/resource/evidence/assessment_outcrossing_maize.pdf (accessed 08.04.2010)

[36] ScienceDaily. (2007) GM field trials 'underestimate potential for cross-pollination. http://www.checkbiotech.org/green_News_Genetics.aspx?infoId=14816

[37] Rieger, M.A., Lamond, M., Preston, C., Powles, S.B. & Roush, R.T. (2002). Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* 296: 2386-2388

[38] Devos, Y., Reheul, D. & De Schrijver, A. (2005). The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross-fertilization. Environ Biosafety Res 4: 71-87.

[39] Biosafety Assessment Tool, GenØk - Centre for Biosafety, http://bat.genok.org/bat/, (accessed 09.04.2010)

[40] Ministry of Science and Technology. Government of India. Biology of Maize. http://dbtbiosafety.nic.in/guidelines/maize.pdf (accessed 22.06.2011)

[41] Ribaut, J-M, Hoisington, D., Banziger, M., Setter, T. and Edmeades, G. Genetic Dissection of Drought Tolerance in Maize: A Case Study. Personal correspondence by author.

[42] http://www.agassessment.org/ (accessed 09.04.2010)

[43] Raj Patel, Eric Holt-Gimenez & Annie Shattuck. Ending Africa's Hunger. The Nation, September 2 2009. http://www.thenation.com/doc/20090921/patel_et_al (accessed 09.04.2010)