Genetic Engineering Briefing Pack March 2004

Monsanto's GE 'Roundup Ready' Soya – What more can go wrong?

Monsanto's Roundup Ready (RR) soya was one of the first genetically engineered (GE) crops to be commercialised. However, since commercialisation there have been a series of discoveries of irregularities and unexpected effects with the RR sova. In addition, the supposed benefits such as reduced herbicide application, and of the benign nature of the associated herbicide, Roundup. have proved unfounded. Monsanto's RR soya should be withdrawn as there are serious doubts over its environmental safety.

GE organisms are products of a crude technology that are inherently unsafe for the environment. There should be no deliberate environmental releases of GE organisms to the environment.

Introduction

Monsanto's Roundup Ready (RR) soybean was first approved for planting in the USA 1994 and subsequently in Canada, Argentina and Mexico. In 1996, RR soya was grown commercially for the first time by farmers in the US and Argentina. It was granted market approval (for import and processing into nonviable soya bean fractions only) in the EU (1) and in Japan (2) in 1996.

RR soya has been GE to make the plant resistant to the herbicide glyphosate. Glyphosate is marketed under the trade name 'Roundup', which is also manufactured by Monsanto. This means that Roundup can be used to kill weeds in soybean crops without harming the soybean plants themselves.

Consent to allow the growing and/or import of RR soya was based on information provided by Monsanto. Monsanto provided data and information, which should have demonstrated that there are no potentially negative effects arising from the RR soya. Government authorities in several countries information have accepted this from Monsanto and given consent for its commercial use. However, since the mid 1990s there have been a series of discoveries of irregularities and unexpected effects with the RR soya, adding to serious doubts over its environmental safety.

Monsanto's Data - are they reliable?

In order to get authorisation for its GE soya, Monsanto submitted an application dossier to the relevant national authorities in the 1990s. This dossier clearly stated that a single copy of a specific novel genetic insert of DNA was present in its GE RR soya. However, after a series of discoveries, it is now clear that the GE soya contains additional fragments of the genetic insert gene and portions of the plant's own DNA are rearranged.

In May 2000, it came to light that additional fragments of the DNA insert were present. These were not intended to be inserted, but are the result of the crude technique used during the genetic engineering of RR soya. Monsanto submitted reports (3) detailing these additional fragments and claiming that "both DNA segments are non-functional" and hence did not pose a problem.

The reports submitted also gave a supposedly detailed molecular characterisation of the genetic insert and the DNA surrounding the insert, the flanking regions. However, in 2001 a report published by a team of independent scientists on the DNA sequence surrounding the main genetic insert (4), the flanking regions, showed that there are serious errors even in this detailed characterisation submitted by Monsanto (3) in 2000.

Genetic Engineering Briefing Pack March 2004

This scientific report (4) now shows that RR soya contains, not only 2 unintended additional fragments, but also that a segment of DNA adjacent to the primary insert that is unrecognisable. The authors suggest that this DNA could be scrambled plant DNA or a large deletion of plant DNA during integration of the insert, or it could also be a segment of DNA from an unknown source. Monsanto provided further information in 2002 (5) indicating that part of this fragment is soya DNA but is rearranged. A substantial portion (20%) still remains unidentified.

Furthermore, contrary to Monsanto's report in 2000 (3), one of the extra DNA fragments in RR soya and some of the rearranged plant DNA <u>are</u> functional. Monsanto now admit (6, 7) that this DNA is expressed (transcribed) to the intermediary product, RNA, one step away from producing a protein. The fact that there is transcription of this DNA raises the possibility of unexpected, untested novel proteins being produced in the soya.

The "rearranged/unidentified" DNA could also result in unintended and unexpected changes to the protein chemistry of the plant. E.g. if the unidentified DNA is scrambled plant DNA or a large deletion of plant DNA, it may have interrupted part of a sequence that codes for one or more plant proteins. This/these protein(s) may no longer be produced by the plant, or may be produced in a modified form. Any changes in plant protein production induced by the unidentified DNA may be significant but not immediately obvious. Changes might only appear after several generations, or in a time of plant stress.

For example, the rearranged/unidentified DNA could cause non-production or modification of a plant protein produced only in response to environmental stress, such as heat or drought, in order to cope with that stress. In this case, any effects would only be seen under such environmental stress. Indeed, heat stress has been shown to cause stem splitting in GE soya possibly due to increased lignin content, although the exact cause of this is not known (8).

Insert characterisation is a relatively straightforward task; based on methods that has been available for several years. When Monsanto submitted its notification in 1994, with the aim of getting timely EU approval for the first US harvest of GE soya, Monsanto failed to correctly provide even the most basic information about its GE soya.

Providing the correct and accurate information is of relevance for a risk assessment. Any products from expression of this additional fragment and rearranged/unidentified DNA are unknown and untested. The original risk assessment done on the GE soya did not take into account either the additional gene fragments, or the presence and potential function of this newly rearranged/unidentified discovered DNA. Therefore, the risk assessment done in 1994 to 1996 cannot claim to be a valid safety assessment of the GE soya currently being grown and sold. There are important and, as yet, unanswered questions regarding exactly what is in Monsanto's GE RR soya and, indeed, what else remains to be discovered.

Monsanto's promises - are they real?

Monsanto claimed that RR soya yields more that conventional soybeans. However, there has been concern regarding yields as far back as 1997, just after RR soya was first commercially planted. In 1998, several US universities conducted RR soybean trials and found a yield drag of 4 % (9). Scientific analysis, published in 2001, clearly shows that yields of RR soya are suppressed (10), but that this is not due to the spraying of glyphosate on the RR soya (11). These studies demonstrated that "a 5% yield suppression was related to the gene or its insertion process

Genetic Engineering Briefing Pack March 2004

and another 5% suppression was due to cultivar genetic differential". They conclude that "the yield suppression appears to be associated with the Roundup Ready gene or its insertion process rather than glyphosate itself" (10).

The yields of GE RR soya have been conclusively proven in studies over several years to be lower than that of their non-GE counterparts. Therefore, Monsanto's claims of higher yields are not valid.

RR soya – is it safe for the environment?

Herbicide use and impact: Roundup is toxic for almost all plants. The very fact that glyphosate is a broad-spectrum herbicide means that many harmless plant species are destroyed unnecessarily. This may lead to decreases in wild plant diversity with damaging consequences for insects, birds and mammals that are dependent on these plants. For example, the rapid and widespread adoption of GE herbicide-tolerant soy and maize may seriously decrease the populations of milkweed, a common agricultural weed in parts of the US. This, in turn, may lead to a decline in monarch butterflies, as milkweed is the sole food source for the butterfly caterpillars (12). Thus the widespread use of broad spectrum herbicide in this the commercial growing of GE RR crops may have adverse consequences for weed plant species and for biodiversity.

New problems with glyphosate are emerging. It has recently been reported that glyphosate usage in one year may encourage the growth of the fungus, fusarium, on wheat grown the next year (13). Fusarium produces toxins, which are damaging to human and animal health. Restrictions on the use of glyphosate were imposed by the Danish government in June 2003 because it was found that glyphosate was detected in groundwaters, having leached from the soils (14). The use of RR GE crops has lead to increased usage of glyphosate and an increasing number of weeds are now resistant to glyphosate, causing additional herbicides to be increasingly used (15).

Hence, the widespread use of glyphosate is likely to have adverse consequences for biodiversity.

Roundup toxic to beneficial bacterium: Glyphosate (Roundup) applications on RR soya can inhibit soybean root growth and nitrogen fixation especially under water deficient conditions (16). Soya has an important symbiotic relationship with a bacterium, Bradyrhizobium japonicum. This nutrient, bacterium fixes the essential nitrogen, from the atmosphere into soybean root nodules, where it can be taken up into the soya plant. The RR soy plant contains the genetic insert that produces an enzyme making the plant resistant to glyphosate. However, the nitrogen-fixing bacteria do not contain the GE insert and are sensitive to Glyphosate glyphosate. is not readily degraded in soybeans and it concentrates in 'metabolic sinks' such as young roots and developing and mature nodules, where it interferes with this symbiotic relationship and delays nitrogen fixation. There were differences in sensitivity to glyphosate among RR soya cultivars, with biomass decreases in response to glyphosate ranging from 0 to 30% at 40 days after emergence for the most tolerant and sensitive cultivars (16). The GE herbicide tolerant soya concept is fundamentally flawed. It ignores the complex plant-microbe interactions in the soil.

Genetic pollution: Soya can cross with other member of the genus *Glycine*, which are found in Australasia including Japan. Natural hybridisation is known to occur between cultivated soybean and *G. max.* ssp. *max*, a common weed in Japan (17). China is the centre of origin and diversity for soya, with more then 6 000 wild soya varieties, over

Genetic Engineering Briefing Pack March 2004

90% of the global total. There are major risks if the GE soya is grown in places where there are wild related species. In such areas, the GE soya does not even have to be grown intentionally, RR soya plants could grow from RR soybeans released unintentionally or accidentally into the environment. Spillage of RR soybeans is inevitable during transport and handling, even if the soybeans are imported for food use only. As one ecologist commented: "Given that crop seeds travel hundreds kilometres between seed of merchant, farmer and processing factory, spillage in transport is inevitable - and more worrying than pollen spread" (18).

Recently, it has been shown that GE maize has contaminated Mexican traditional maize varieties (19). Mexico is the centre of diversity for maize. In 1998, Mexico imposed a moratorium on the planting of GE maize. One of the sources of the contamination is imported US maize. Approx. 25 percent of US maize is GE, and the US refuses to keep the GE maize segregated from traditional maize. The lack of labelling requirement favours an 'accidental' mix up or use for planting of the imported GE maize. Thus, the import of GE soybeans into a centre of origin and/or diversity for soya, such as China, poses the risk of genetic contamination of valuable soya diversity.

Scientists have raised concern over the potential for crop-to-wild gene flow to lead to the extinction of rare species. This extinction can happen in two ways _ through demographic swamping and genetic assimilation. During swamping, the population of wild plants shrinks in size because crop-wild hybrids are less fertile. Small populations and rare species can be lost. The second process is known as genetic assimilation, where crop genes replace the genes in wild species through continual hybridisation (20). Recent research found that genes from transgenic crops could rapidly take over those in wild relatives (21). The combination of the forces of swamping and genetic assimilation could then lead to what evolutionary biologists call a "migrational meltdown"(20).

Monsanto's risk assessment– is it sound?

Monsanto's 'safety' assessment of its genetically engineered soybean uses the principle of 'substantial equivalence' (22). The use of substantial equivalence in the regulatory process has been the subject of controversy since its introduction (23). A comprehensive study by the Royal Society of Canada (24) has seriously undermined usage of the concept. The Canadian report states that current regulatory use of substantial equivalence uses a "decision threshold" interpretation. This interpretation assumes that no changes occur in the plant other than those directly attributable to the inserted gene: the food can be considered to be equivalent to "natural" counterpart after routine its chemical analysis, normally only of major constituents and those known to be potentially toxic, e.g. solanines in potato varieties. This is in contrast to a "safety standard" interpretation (recommended by the Royal Society of Canada) which would require rigorous scientific analysis to assess (and possibly attribute) all and each of the effects created by genetic engineering.

Most of the data in the field tests in Monsanto's application for marketing under are visual EU legislation 90/220/EEC observations made by breeders, which would only detect serious visible unintended effects. No physiological or biochemical parameters analysed were (e.g. nitrogen uptake. photosynthesis rate). Neither the effects of the genetic modification on the whole plant, nor the correct genetic functioning of the plant were assessed. It is clear that the possible risks have NOT been sufficiently studied and assessed.

Genetic Engineering Briefing Pack March 2004

Any changes in protein chemistry that do not lead to immediately apparent or visible changes, but are nonetheless significant, would not have been detected in the original application for marketing and food safety assessment. These assessments would only have detected major differences between modified and unmodified soya in terms of agronomic performance and nutritional analyses.

example, since the For food safety assessment, differences in phytoestrogen levels between GE and non GE soya have been found (25) which were not documented in the original food safety assessment. These phytoestrogens are believed to be of clinical importance. There is growing interest and use of soybean-based food products or extracts to increase dietary phytoestrogen intake (22). Whether or not there are further problems with the GE soya is unknown.

Conclusions

There important and, are as vet, unanswered questions regarding exactly what is in Monsanto's RR soya and what else remains to be discovered. The risk assessment done in 1994 to 1996 cannot claim to be a valid safety assessment of the GE soya currently being grown and sold. The yields of RR soya have been proven to be lower than that of their non-GE counterparts, invalidating Monsanto's claims of higher yields. The widespread use of glyphosate is likely to have adverse consequences for biodiversity. The import of GE soya into a centre of origin and/or diversity for soya poses the risk of genetic contamination of valuable soya diversity. The GE herbicide tolerant soya concept is fundamentally flawed as it ignores the complex plant-microbe interactions in the soil. Monsanto's RR soya should be withdrawn, there are serious doubts over its environmental safety.

GE organisms are products of a crude technology. They are likely to produce unexpected and unpredictable effects and therefore are inherently unsafe for the environment. Once released, GE organisms cannot be recalled because they are biological organisms capable of selfreproduction. Therefore, there should be no deliberate environmental releases of GE organisms to the environment.

REFERENCES

- (1) Commission Decision of 3 April 1996 concerning the placing on the market of genetically modified soya beans (*Glycine max* L.) with increased tolerance to the herbicide glyphosate, pursuant to Council Directive 90/220/EEC (96/281/EC).
- (2) According to the OECD database, RR soya was first approved in the USA for planting and use in 1994, it is approved for planting and use as well in Canada, Argentina and Mexico, for use but not for planting in the EU, Japan and Switzerland. This list may be incomplete.
- (3) Dossier from Monsanto containing molecular analysis of RR soya: http://archive.food.gov.uk/pdf_files/acnfp/summary.pdf http://archive.food.gov.uk/pdf_files/acnfp/dossier.pdf. Available at: http://www.foodstandards.gov.uk/science/ouradvisors/n ovelfood/assess/assess-uk/60500/
- (4) Windels, P., Taverniers, I. Depicker, A. Van Bockstaele, E. & De Loose, M. (2001) Characterisation of the Roundup Ready soybean insert. European Food Research Technology, 213:107-112.
- (5) Monsanto (2002a) DNA Sequences Flanking the 3' End of the Functional Insert of Roundup Ready Soybean Event 40-3-2 and Transcript Analysis of the Sequence Flanking the 3' End of the Functional Insert in Roundup Ready Soybean Event 40-3-2. Available at: http://www.food.gov.uk/multimedia/webpage/72699.
- (6) Monsanto (2002b) Transcript Analysis of the Sequence Flanking the 3' End of the Functional Insert in Roundup Ready Soybean Event 40-3-2. Available at: http://www.food.gov.uk/multimedia/webpage/72699.
- Monsanto (2002c) 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.foodstandards.gov.uk/multimedia/pdfs/RRS safetysummary.pdf
- (8) Coghlan, A. (1999) Splitting headache. Monsanto's modified soya beans are cracking up in the heat. New Scientist, 20 Nov. 1999, p. 25.
- (9) Benbrook, C. (2001) Troubled times amid commercial success for Roundup Ready soybeans.



Genetic Engineering Briefing Pack March 2004

http://www.biotech-info.net/troubledtimes.html and Oplinger, E.S., Martinka, M.J. Schmitz, K.A. (1999) Performance of transgenetic soybeans – northern US. http://www.biotech-info.net/herbicidetolerance.html#soy

- (10) Elmore, R.W., Roeth, F. W., Nelson, L.A., Shapiro, C.A., Klein, R.N., Knezevic, S.Z. & Martin A. (2001) Glyphosate-resistant soybean cultivar yields compared with sister lines. Agronomy Journal, 93: 408-412.
- (11) Elmore, R.W., Roeth, F.W., Klein, R.N., Knezevic, S.Z., Martin, A., Nelson, L.A. & Shapiro, C.A. (2001) Glyphosate-resistant soybean cultivar response to glyphosate. Agronomy Journal, 93: 404-407.
- (12) B. Hartzler (1999) Monarch butterflies and herbicide resistant crops. Iowa State University, www.weeds.iastate.edu/weednews/monarchs.htm and "Monarch butterflies may be threatened in their North American range," Environmental Review 6(4):1-9, April 1999 and "Monarchs and Their Roots" Science, 283: 171.
- (13) Coghlan, A. (2003) Weedkiller may encourage blight. New Scientist, 16th August 2003, pg. 6.
- (14) www.pesticidvarsling.dk, www.geus.dk, http://www.mim.dk/nyheder/presse/Dep/040603_glypho sat.htm
- (15) Greenpeace (2004) More and more "superweeds" with genetically-engineered crops. Greenpeace Briefing Paper. www.greenpeace.org.
- (16) King, C.A, Purcell, L.C, & Vories, E.D, (2001) Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to foliar glyphosate applications. Agronomy Journal, 93:179-186.
- (17) Nakayama, Y. & Yamaguchi, H (2002) Natural hybridization in wild soybean (*Glycine max ssp. soja*) by pollen flow from cultivated soybean (*Glycine max ssp. max*) in a designed population. Weed Biology and Management, 2: 25–30.
- (18) Crawley, M, (1996) The day of the triffids. New Scientist, 6 July, 40-41.
- (19) Quist, D. and Chapela, I.H. (2001) Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. Nature, 414: 541-543
- (20) Ronce, O. and Kirkpatrick, M. 2001. When sources become sinks: Migrational meltdown in heterogeneous habitats. *Evolution* 55: 1520-1531.
- (21) Haygood, R., A.R. Ives, and D.A. Andow. 2003. Consequences of recurrent gene flow from crops to wild relatives. Proceedings of the Royal Society of London B, Biological Sciences, 270: 1879 – 1886.
- (22) see Application to the UK Advisory Committee on Novel Foods and Processes for Review of the Safety of Glyphosate Tolerant Soybeans by the Agricultural Group of Monsanto Company, July 27 1994.
- (23) Millstone, E., Brunner, E. & Mayer, S. (1999) Beyond "substantial equivalence". Nature, 401, 525-526.
- (24) Royal Society of Canada. Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada (2001).
- (25) Lappé, M.A., Bailey, E.B., Childress, C.C. & Setchell, K.D.R. (1998/1999), Alterations in Clinically Important Phytoestrogens in Genetically Modified, Herbicide-Tolerant Soybeans, Journal of Medicinal Food, 1:241-245.