Roundup Ready Soya:
Incomplete data, missing evaluation and insufficient controls

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Summary

Monsanto's genetically modified or genetically engineered (GE) Roundup Ready (RR) soya was first approved for planting in the USA 1994 and subsequently in Canada, Argentina and Mexico. It was granted market approval (for import and processing into non-viable soya bean fractions only) in the EU in 1996[1] and in Japan[2]. Consent was granted based on the RR soya containing a single copy of a specific novel gene insert.

Subsequently, in May 2000, it came to light that additional fragments of the foreign DNA insert were present. Monsanto submitted a report[3] detailing these additional fragments and claiming that these fragments were unlikely to be functional and hence did not pose a problem. Relevant EU authorities have thus far avoided the question of the illegality of Monsanto selling a product that contains gene inserts that were never approved.

Now, a new scientific report[4] shows that this RR soya contains an additional segment of DNA adjacent to the primary insert that is unrecognisable. It could be scrambled plant DNA or DNA from unknown sources. The function of this unidentified DNA is unknown, untested and unapproved.

Monsanto failed to provide precise and complete information regarding the characterisation of the insert of RR soya when filing its application for market approval in 1994. Monsanto failed to disclose the presence of two additional fragments of the insert and the presence of unidentified DNA. Crucial information such as the source and function of the unidentified segment of DNA is still missing.

The GE soya currently being planted in the USA, Canada and Argentina and exported as food and animal feed does not genetically match that granted marketing consent in the EU. The original risk assessment for this GE soya has not taken into account these additional inserts.

Presence of additional inserts – May 2000

Recently, a Belgian scientist, Dr. Marc De Loose, and his team developed a new precise, fast and inexpensive method to characterise the DNA of the inserts of GE crops and the regions of plant DNA flanking the inserts. When he examined Monsanto's RR soya, Dr. De Loose discovered that his genetic map did not match the RR soya that had actually been approved in the EU: two additional fragments of the insert were present in the plant. These additional inserts comprised a 250 base pair[5] fragment (of the CP4 EPSPS gene) adjacent to the principal insert and a 72 base pair fragment (also of the CP4 EPSPS gene) as a second insert. Dr. De Loose informed Monsanto and the Belgian authorities about his discovery. Monsanto eventually informed the UK[6] competent authority, the Food Standards Agency (FSA) on 18 May 2000[7].

Unidentified DNA – August 2001

New information on the sequence of the DNA either side of the principal insert has just been published in a peer-reviewed scientific journal[3] by a team led by the same scientist, Dr. De Loose. This study has found further serious abnormalities with the DNA in RR soya.

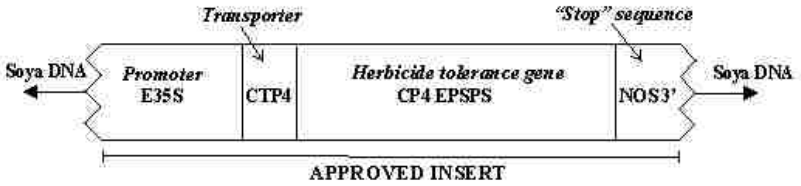
In response to the discovery of the first additional inserts, Monsanto submitted a report in May/June 2000[2] to the relevant committee of the UK FSA, the Advisory Committee on Novel Foods and Processes (ACNFP). This report gave detailed characterisation of both the additional inserts and the sequence of the plant DNA either side of the inserts (the flanking or junction regions). The Monsanto report sequenced the flanking region beyond the additional 250 base pair fragment adjacent to the principal insert. This DNA sequence was labelled as "soybean genomic DNA". In their report, Monsanto did not give any information on how their conclusion of "soybean genomic DNA" was reached, nor of how the unmodified soya control was used to verify this result.

Dr. De Loose obtained the same sequence (99 % similarity) for this DNA flanking region but then demonstrated that this region of alleged "soybean genomic DNA" was not present in the unmodified soya plant. The sequence presented by Dr De Loose went further than the 415 base pairs of the flanking region in the Monsanto report and only reached recognisable soya plant DNA after 534 base pairs (see Figure).

This 534 base pair sequence does not match with any known DNA. 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. Therefore, the question as to what exactly is in Monsanto's soya remains unanswered.

Schematic Overview

a) Approved DNA insert as described by Monsanto in their original EU application for marketing (from Monsanto, 2000)[3]. The function of each individual component of the insert is stated in italics.



b) Unapproved, multiple DNA inserts and unidentified DNA as now revealed (unapproved DNA is shaded). Two additional, unapproved inserts are present: a 250 base pair (bp) fragment of CP4 EPSPS attached to the main insert and a separate 72 bp insert of CP4 EPSPS (Monsanto, 2000)[3]. Adjacent to the unapproved 250 bp insert is the newly discovered (Windels et al. 2001)[4] 534 bp of unidentified, unapproved DNA.

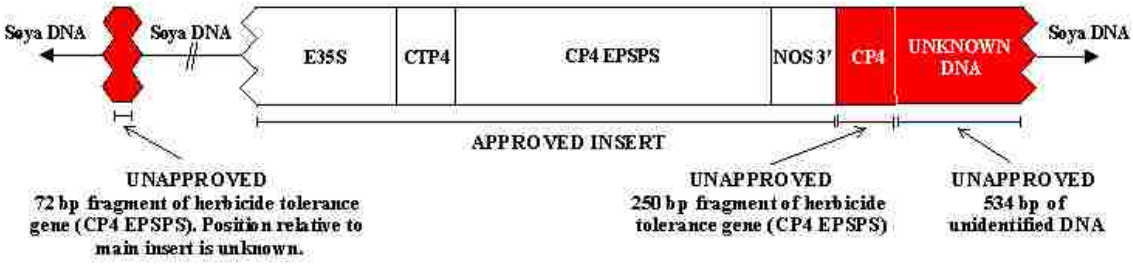


Figure. Schematic of the DNA inserts in Monsanto's Roundup Ready soya. Abbreviations: bp - base pair, used to indicate the length of the DNA fragments[3]; E35S - cauliflower mosaic virus promoter; CTP4 - chloroplast transit peptide sequence from petunia; CP4 EPSPS - herbicide tolerance gene from *Agrobacterium sp.*, strain CP4; NOS 3' - nontranslated region of nopaline synthase gene. For footnotes see main text.

Any function that this segment of DNA may have played or does now play is, as yet, completely unknown. If the unidentified DNA is scrambled plant DNA it may have interrupted part of a sequence that codes for a protein. It may have created what is known as an "open reading frame" by scrambling a 'stop' part of a protein-coding DNA sequence or by introducing a new 'start' codon (i.e. disruption of the signal for when to start or when to stop the gene function). The unknown DNA itself could code for a novel protein - approximately 80-85 % of plant protein coding units (exons) are less than 500 base pairs long[8], i.e. smaller than the size of the unknown DNA sequence.

Even if the unknown DNA occurs in a non protein-coding region of plant DNA, its presence could interfere with the normal expression of plant genes by affecting the regulatory network, the molecular basis of which is still largely unknown[9].

It is difficult to speculate upon what the actual outcome in terms of human and animal health, environment or agronomy may be since the very basic information about this newly discovered region of Monsanto RR soya is not known and it is unknown whether any further unintended changes have occurred anywhere else in the soya genome.

However, issues such as differences in phytoestrogen levels[10], increased lignin content, which made RR soya plants brittle in hot temperatures[11] and reduced yields from RR soya[12] have previously been raised but never fully explained.

The UK ACNFP was informed of this unidentified DNA in November 2000[13] by the Belgian scientists[14] and discussed it at the ACNFP January 2001 meeting[15] where it was agreed that there was still some uncertainty regarding the origin of the DNA fragment. The committee has asked Monsanto to provide data demonstrating that this DNA is 'silent' and does not result in the production of a novel protein.

Crucial information, such as the source of the unidentified segment of DNA and possible functions of this segment is still missing. It is still not known whether any new proteins are formed nor whether the genetic insertions have caused interference with normal plant metabolism.

The publication of this sequence of unidentified DNA in a peer-reviewed scientific journal has brought this matter to light. The discovery was not brought to the authorities' attention by Monsanto. This seriously undermines assurances by GE companies that they have either the required knowledge or understanding of the techniques they employ and also raises serious doubts that the regulatory authorities insist on scientific quality and accuracy.

What was approved and what was not approved in the EU

According to Directive 90/220/EEC (article 11.1, Annex II.II.C 1&2), characterisation of the insert is one of the fundamental pieces of information to be provided by notifiers in order to obtain market approval in the EU, and both risk assessment and market approval are done on a case by case basis for each GE organism. Market approval is only granted for that GE organism with the specified genetic arrangement described. A GE organism that contains additional genetic material and/or changed genetic arrangement requires a new assessment and separate approval.

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.

The genetic characteristics of the inserts contained in the GE soya that is currently imported in the EU are different from the GE soya that was approved in 1996. The GE soya currently being imported and used in food and feed is a new GE organism containing additional sequences and unidentified DNA.

The EU Commission Decision of 3 April 1996 (96/281/EC) states:
"…consent shall be given by the competent authorities of the United Kingdom for the placing on the market of the following product notified by Monsanto Europe (Ref.

C/UK/94/M3/1) under Article 13 of Directive 90/220/EEC. The product consists of soya beans derived from a soya bean (*Glycine max* L. cv A5403) line (40-3-2) in which the following sequences have been inserted:

- a single copy of the gene coding for glyphosate tolerance CP4 5 enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) from *Agrobacterium* sp. strain CP4, and the chloroplast transit peptide (CTP) coding sequence from *Petunia hybrida* with the promoter P-E35S from cauliflower mosaic virus and the nopaline synthase gene terminator from *Agrobacterium tumefaciens".*

The Roundup Ready soya currently being sold contains additional gene sequences. A second 72 base pair DNA fragment (from CP4 EPSPS) was found and an additional 250 base pair segment of CP4 EPSPS DNA was also identified. This latest discovery shows a further 534 base pair segment of `unidentified DNA`.

In May 2000 (4 years after appearing on the EU market), additional data was submitted by Monsanto to the UK ACNFP regarding the first two additional gene sequences, including a dossier giving a supposedly detailed molecular characterisation of the insert and flanking regions[2]. However, the newly published DNA sequence[3] shows that there are serious errors even in the detailed characterisation submitted by Monsanto.

The RR soya currently being sold in Europe is clearly not genetically the same as that granted consent. The original risk assessment conducted on Monsanto's RR soya did not take into account the newly discovered `unidentified DNA`. Therefore, the RR soya currently being sold has never actually been granted consent and the risk assessment that was based on this RR soya must now be deemed invalid.

Before Monsanto's new version of Roundup Ready soya can be put on the market, a new application should be submitted by Monsanto under the old Directive 90/220/EEC (or under the new Directive 2001/18/EC) and under the Novel Food regulation, 258/97/EC.

Conclusions

1) Monsanto have not provided or even apparently been aware of the correct basic scientific information regarding the genetic make-up their genetically engineered Roundup Ready Soya.
2) The GE soya currently on sale in Europe is not that which was approved. The original risk assessment done on the GE soya did not take into account either the additional gene fragments nor the presence and potential function of this newly discovered `unidentified DNA`. Therefore, the risk assessment done in 1994 to 1996 cannot claim to be a valid safety assessment of the GE soya currently being sold.
3) UK and EU Authorities must now take action to uphold their own laws on GE organisms. They should insist that this RR soya is withdrawn from the market and RR soya imports halted until Monsanto have properly applied for and been granted approval for this particular GE organism under all relevant legislation.

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). The decision directs the UK government to grant the EU market consent for Monsanto's RR soya.

[2] According to the Database of the OECD 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://www.foodstandards.gov.uk/pdf_files/acnfp/dossier.pdf, available at http://www.foodstandards.gov.uk/committees/acnfp/acnfpassessments.htm

[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*, (in press). [Published on line 16th May 2001, DOI 10.1007/s002170100336.]

[5] A base pair (bp) is part of the basic unit of DNA. The number of base pairs is commonly used to indicate the length of a DNA fragment.

[6] The country where the notification for approval of RR soya was submitted, which granted it the EU consent.

[7] http://www.foodstandards.gov.uk/committees/acnfp/letter.htm

[8] Lewin, B. (2000) *Genes VII*. Oxford University Press, Oxford, Ch.2, p. 54.

[9] Lewin, B. (2000) *Genes VII*. Oxford University Press, Oxford, Ch. 2, p. 63.

[10] 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.

[11] Coghlan, A. (1999) Splitting headache. Monsanto's modified soya beans are cracking up in the heat. New Scientist, 20 Nov. 1999, p. 25.

[12] Benbrook, C. (2001) Troubled Times amid Commercial Success for Roundup Ready Soybeans. Available at http://www.biotech-info.net/troubledtimes.html

[13] Minutes of the 47th meeting of the ACNFP, 16th November 2000: http://www.foodstandards.gov.uk/committees/acnfp/minutes/001116.htm and ACNFP Annual Report 2000. Published 30th April 2001: http://www.foodstandards.gov.uk/pdf_files/acnfp/acnfp00.pdf

[14] Papers for the 48th meeting of the ACNFP, 25th January 2001: http://www.foodstandards.gov.uk/pdf_files/acnfp/acnfp_48_5papers.pdf

[15] Minutes of the 48th meeting of the ACNFP, 25th January 2001: http://www.foodstandards.gov.uk/committees/acnfp/minutes/010125.htm

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