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TIME FOR THE EU TO REJECT
Monsanto's Genetically Engineered 'Roundup Ready' Soya

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Introduction

Monsanto's Roundup Ready soya was one of the first genetically engineered (GE) crops to be commercialised in the mid 1990s. In the EU, Monsanto's GE Roundup Ready soya has been authorised for import and processing in food and feed since 1996. In 2006 the ten year licensing authorisation expired and Monsanto had to apply for a renewal. This time, Monsanto applied in the EU to be granted authorisation not only for import as food and feed but also for cultivation in European fields.

In this paper, Greenpeace raises a series of serious scientific concerns calling for a rejection of the entire application of Monsanto's GE Roundup Ready soya.

- GE Roundup Ready soya cannot be considered to be as safe for human and animal as its non-GE counterpart because, since its commercialisation, there have been a series of discoveries of irregularities and unexpected effects with this product.
- GE Roundup Ready crops are harming the environment where they are grown: the supposed environmental benefits of GE Roundup Ready soya such as reduced herbicide application, and of the benign nature of the associated herbicide, Roundup, have proved unfounded. Instead, Roundup-tolerant weeds are increasing causing more and more herbicides to be applied.

For the above reasons Greenpeace is calling on the European Commission and EU member- states to reject the Monsanto GE Roundup Ready soya application.

Concerns raised as new discoveries found with Monsanto's GE Roundup Ready soya since commercialisation

Monsanto's Roundup Ready soya has been genetically engineered (GE) to make the soya tolerant to the herbicide glyphosate, which is also manufactured by Monsanto and sold as Roundup. GE Roundup Ready soybean was one of the first GE crops ever grown commercially. It was approved for planting in the USA 1994 and subsequently in Canada, Argentina, Brazil and Mexico. In 1996, it was granted market approval in the EU for ten years for import and processing only, the application was not for cultivation[1]. Now, the ten years are up and Monsanto has applied for GE Roundup Ready soya to be re-approved[2]. This requires the GE Roundup Ready soya to be re-assessed. Importantly, the new application includes a

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request for cultivation. Thus, the European Commission must also consider the environmental implications of cultivating GE herbicide tolerant soya.

The original application was heavily criticised, including criticism from Greenpeace for the poor quantity and quality of the data submitted. Unbelievably, in Monsanto's application, the soya had not been sprayed with Roundup. The original 1996 risk assessment was wholly inadequate and should be disregarded in the renewal application.

Since EU approval in 1996, there have been several discoveries casting doubt on its environmental, food and feed safety. These provide ample evidence that GE Roundup Ready Soya certainly should not be cultivated anywhere, and shouldn't be used as food for humans or animals. These discoveries are not highlighted in Monsanto's application for renewal, but are described here as grounds for rejection of the renewal of approval of Monsanto's RR soya in the EU.

Discoveries casting severe doubt on the safety of Monsanto's GE Roundup Ready soya for food/feed and the environment since 1996 include:

- The build-up of weed tolerance to the herbicide used with GE Roundup Ready soya requires increasing amounts of herbicide and more powerful herbicides to be applied and
- GE Roundup Ready soya contains additional fragments of the genetic insert and the crude genetic engineering method has caused rearrangements of plant DNA, possibly producing unintended proteins.

1) Environmental problems from growing GE Roundup Ready soya

It's becoming increasingly apparent that cultivation of GE Roundup Ready soya is causing environmental problems where it is grown. More and more herbicide is needed as weeds become tolerant to the Roundup herbicide and new research shows that Roundup is not as environmentally friendly, or benign, as previously thought.

Roundup toxicity and persistence

GE Roundup Ready soya is tolerant to the herbicide glyphosate, the active ingredient in 'Roundup', which is also manufactured by Monsanto. Monsanto claim that glyphosate is a relatively benign herbicide[3]. However, Roundup contains other chemicals in addition to the active ingredient, glyphosate. Some of these are surfactants, which make the glyphosate adhere to plant leaf surfaces so it is taken up into the plant. Although the additional chemicals are not always known, it is now becoming clear that they significantly increase the toxicity of Roundup formulations compared with the active ingredient glyphosate. It has been shown that Roundup is toxic to tadpoles, affecting aquatic communities, reducing biodiversity[4] and at least one formulation of Roundup has been shown to be a potential endocrine disrupter, i.e. could interfere with hormones[5].

Glyphosate may not be as readily degradable initially claimed. It has been shown to leach into Danish soils where it could "*pose a potential risk to the aquatic environment*"[6].

In addition, glyphosate can encourage fungal infections in crops. It has been reported that glyphosate usage in one year may encourage the growth of the fungus, fusarium, on wheat grown the next year[7]. Fusarium produces toxins, which are damaging to human and animal health and cause economic losses to farmers.

Hence, Roundup may not be as benign as first appears and its usage is likely to have adverse consequences for biodiversity and possibly also for farmworkers who come into close contact with the herbicide.

More and more herbicides

The widespread use of glyphosate is associated with Roundup GE crops (predominantly soya but also including GE Roundup Ready corn and cotton) since their introduction a decade ago. This has lead to the creation of glyphosate-tolerant weeds. This, in turn has lead to increases in the amount of glyphosate and the use of more powerful herbicides.

The general mechanisms that lead to the creation of glyphosate-tolerant weed populations are well known. They start as individual plants whose genetic make-up differ slightly and can, as a result, survive an attack by a herbicide, either by excluding the herbicide (resisting) or tolerating it. They exist at first in small numbers but the frequent application of the herbicide supplies a selection pressure, enabling these herbicide-resistant/tolerant plants to survive better than non-tolerant plants, and hence spread at the expense of the non tolerant/resistant plants[8].

In the US, glyphosate-tolerant weeds are occurring in direct association with Roundup GE crop cultivation. The most frequently found and widespread is horse-weed or marestail (*Conyza canadensis*). First discovered in Delaware in 2000, this horseweed can withstand 8-13 times as strong a dose of the herbicide[9]. Reports of glyphosate-tolerant horseweed have steadily increased until, by the end of 2005, glyphosate-tolerant horseweed has been reported in thirteen US states[10]. Similarly, populations of waterhemp (*Amaranthus rudis*), which cannot be combated by the usual amounts of glyphosate, have now been found in Iowa, Illinois and Missippi (where they occur in soya fields)[11].

In the US, a massive increase in the amount of glyphosate applied on GE Roundup Ready soya per acre between 1996 and 2004 has been recorded, including a 22 per cent jump between 2000 and 2001[12]. The increase in glyphosate is, at least partially, caused by the emergence of glyphosate-tolerant weeds requiring more and more of the active ingredient, glyphosate, to be applied. In addition, other, more notorious herbicides are now being advertised to control glyphosate-tolerant weeds. For example, it is now recommended that farmers use the notorious 2, 4-D to control glyphosate-tolerant marestail[13].

In Argentina, GE Roundup Ready soya is causing an environmental crisis. In addition to causing deforestation, monocultures of GE Roundup Ready soya have led to massive increases in glyphosate usage. The introduction of GE Roundup Ready soya has lead to a 56-fold increase in total glyphosate use on soybeans in Argentina over the six years to 2005, at least partly as a result of the increase in glyphosate in herbicide formulas[14]. New weeds, tolerant to glyphosate are replacing the usual weeds found in the fields in Argentina[15] and soil micro-organisms are thought to be affected as a result of so much herbicide being applied[16].

In the recent World Trade Organisation (WTO) trade dispute, the European Communities' (EC) scientific arguments included the effects of glyphosate herbicide on soil microorganisms:

"Some data however, do emerge from the use of glyphosate resistant soybeans in the US and some of these findings do rather point in the direction of a change in soil

microbial activity towards favouring fungi over bacteria. For example Kremer et al. (2000) found that in soils repeatedly treated with glyphosate and grown to glyphosate resistant soybeans, soybeans significantly fell victim to a Fusarium fungus causing 'damping off'. It would in fact be rather surprising if such intensive use of one chemical would NOT cause a change in the microbial communities. The experience from Canada and the US also clearly show that the use of the respective herbicides complementary to GM HT crops do increase significantly with the production of the respective HT crops." [17]

Effects on wild plant species

The cultivation of Roundup-tolerant soybeans involves the use of broad-spectrum glyphosate-based herbicides such as Roundup. Glyphosate kills plants indiscriminately, leaving just the GE herbicide tolerant crops and weeds intact. The many wild plants that have not or cannot develop tolerance are destroyed. This could lead to decreases in wild plant density and diversity. This would have damaging consequences for insects, birds and mammals that depend on these plants for food and/or shelter. Such decreases in wild plants were shown for GE herbicide tolerant oilseed rape and sugarbeet (but not maize, and soya was not studied) in the UK, compared to their non-GE counterparts and that there could be important long-term environmental effects[18]. Thus, the widespread use of this broad spectrum herbicide in the commercial growing of GE Roundup Ready crops may have adverse consequences for weed plant species with knock-on effects for biodiversity.

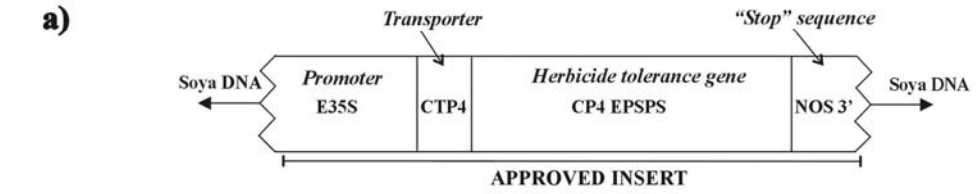
Several problems with glyphosate and Roundup are emerging: herbicide-tolerant weeds, the toxicity and persistence of glyphosate, as well as possible decline in plant diversity. Adverse impacts of the use of Roundup and glyphosate are associated with the use of GE Roundup Ready crops, especially GE Roundup Ready soya. If GE Roundup Ready soya is approved for cultivation in the EU, those countries will suffer from the environmental problems that the cultivation of GE Roundup Ready soya has brought to the USA, Canada and Argentina. These cases are grounds for rejection of the cultivation part of the renewal application.

2) Food/feed safety compromised by unidentified DNA

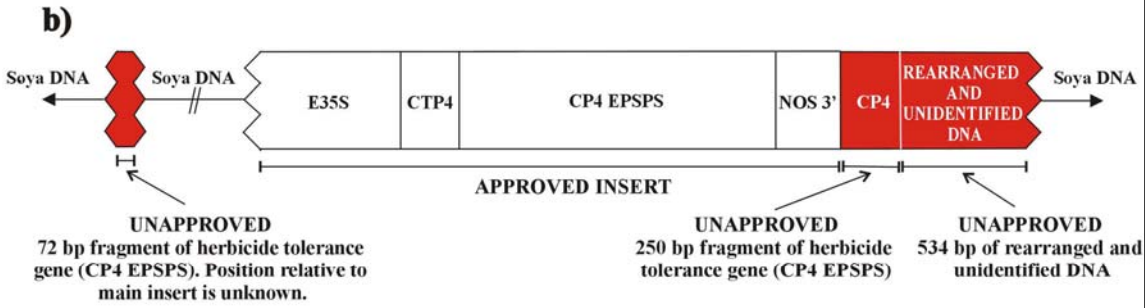
It is now clear that the GE Roundup Ready soya contains additional fragments of the genetic insert gene and portions of the plant's own DNA are rearranged. Disturbingly, it's considered possible that some of these fragments and rearrangements could be made into new, unknown proteins.

In 2001, a publication by a team of independent scientists on the DNA sequence surrounding the main genetic insert[19] showed 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 (see Fig. 1). Monsanto provided further information in 2002[20] indicating that part of this fragment is soya DNA but is rearranged. A substantial portion (20 per cent) still remains unidentified.

Figure: 1. Monsanto's Roundup Ready soya contains unapproved, unintended fragments, rearrangements and unidentified DNA. Abbreviations: bp - base pair; 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.



Approved DNA insert as described by Monsanto in their original EU application for marketing. The function of each individual component of the insert is stated in italics.



Actual DNA inserts including fragments, unidentified DNA (unapproved DNA is shaded). Two additional, unapproved inserts are present: a 250 base pair (bp) fragment attached to the main insert and a separate 72 bp insert at an unknown location in the soya genome. In addition, adjacent to the unapproved 250 bp insert is a region of rearranged and unidentified DNA discovered by independent scientists.

Importantly, one of the extra DNA fragments in GE Roundup Ready soya and some of the rearranged plant DNA <u>are</u> functional. Monsanto themselves admit[21] that this DNA is functional (transcribed) and produces the intermediary product, RNA, one step away from producing a protein. Indeed, it has been shown that the unintended RNA from one of the additional fragments is further processed by the plant to create RNA fragments, called "variants". The authors state "*These [RNA] variants might code for as yet unknown CP4 EPSPS fusion proteins.*" That is, it is possible that unexpected, untested novel proteins could be produced in GE Roundup Ready soya. The food safety of any novel proteins would be completely unknown – they could turn out to be toxic or cause allergies.

In addition to creating novel proteins, 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.

There are important and, as yet, unanswered questions regarding exactly what is in Monsanto's GE Roundup Ready soya, what the additional, unintended genetic inserts and rearranged DNA produce or interfere with. Indeed, it is highly possible more will be discovered, if the research is performed. However, as outlined below the

safety assessment for GE Roundup Ready soya is flawed and in its current state it is unlikely that any unexpected novel protein, or alterations to existing plant proteins, would be detected in the risk assessment. Unsurprisingly, these unknowns are not highlighted in the dossier submitted to the EU in support of the re-approval application, and no further studies on the possible effects of these fragments and rearrangements have been conducted by Monsanto. Hence, the food, feed and environmental safety of Monsanto's GE Roundup Ready soya are completely unknown. If there were long term health effects in humans or animals, these are likely to go unnoticed because of a lack of monitoring.

Flawed Safety Assessment

For the renewal of Monsanto's 'safety' assessment of its genetically engineered soybean the principle of 'substantial equivalence' is used. The use of substantial equivalence in the regulatory process has been the subject of controversy since its introduction[22]. The Royal Society, in the UK, and the Royal Society of Canada have recommended that substantial equivalence should require that any differences found between the GE and non-GE counterpart are thoroughly investigated[23]. However, in practice the companies are rarely, if ever, called to explain significant differences[24]. Hence, the fact that the original risk assessment relied on data from GE Roundup Ready soya plants that included the additional fragments and rearrangements, give no cause for comfort.

It is unlikely that any unexpected novel protein, or alterations to existing plant proteins, would be detected in the risk assessment for two reasons. Firstly, finding changes in protein composition or structure requires detailed analysis, often looking for the unknown. The compositional testing is absolutely minimal and would only have detected major differences in agronomic performance and nutritional analyses between GE and non-GE soya. For example, since the original assessment, differences in phytoestrogen levels between GE and non-GE soya have been found[25]. These phytoestrogens are believed to be important in human health but the differences were not identified in the original compositional analysis Secondly, any changes in plant protein production induced by the unidentified DNA may not be immediately apparent or show visible changes, but could be nonetheless significant. Changes might only appear after several generations, or in a time of plant stress. Indeed, heat stress causes stem splitting in GE soya, possibly due to increased lignin content[26].

Changes in composition, especially with regards to proteins are highly important for food/feed safety. Even small changes in proteins can fundamentally alter their allergenicity or toxicity. Although initial testing did not indicate allergenicity[27], this does not preclude long-term effects. Preliminary research shows some changes at the microscopic level in the cells of internal organs of mice fed GE soya[28] and there are reports of adverse effects on their reproduction[29]. More research is needed to fully evaluate the significance and implications of these findings but this underlines the failings of the risk assessment to detect any subtle changes that could be important. Such changes would be hard to identify unless any adverse effects occur in the general population due to a lack of adequate monitoring[30].

The presence of additional fragments and rearrangements and unidentified DNA severely compromises any concept of the safety of GE Roundup Ready soya for human food and animal feed. These unintended products of the genetic engineering process could alter soya chemistry. Further, the risk assessment is fundamentally flawed and unlikely to detect any subtle or temporal changes that could lead to adverse effects, especially with the

minimal data submitted. It can not be claimed that GE Roundup Ready soya is as safe as its non-GE counterpart because of the additional fragments and rearrangements. Therefore, the application for the renewal of marketing approval of Monsanto's GE Roundup Ready soya should be rejected.

Conclusions

The widespread use of the herbicide glyphosate as Roundup in association with GE Roundup Ready soya in Europe will, most likely, have adverse consequences for biodiversity because of glyphosate toxicity and creation of glyphosate-tolerant weeds. There are important and, as yet, unanswered questions regarding exactly what is in Monsanto's GE Roundup Ready soya and what else remains to be discovered. Like all GE crops, Roundup Ready soya is a product of an outdated, crude and old fashioned technology. The capacity for unexpected and unpredictable effects is increased by the irregularities in the inserted and unknown DNA in GE Roundup Ready soya, combined with the flawed risk assessment. Any competent assessment would reject the renewal application on the grounds that there are negative impacts on the environment and serious doubts about the food/feed safety.

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