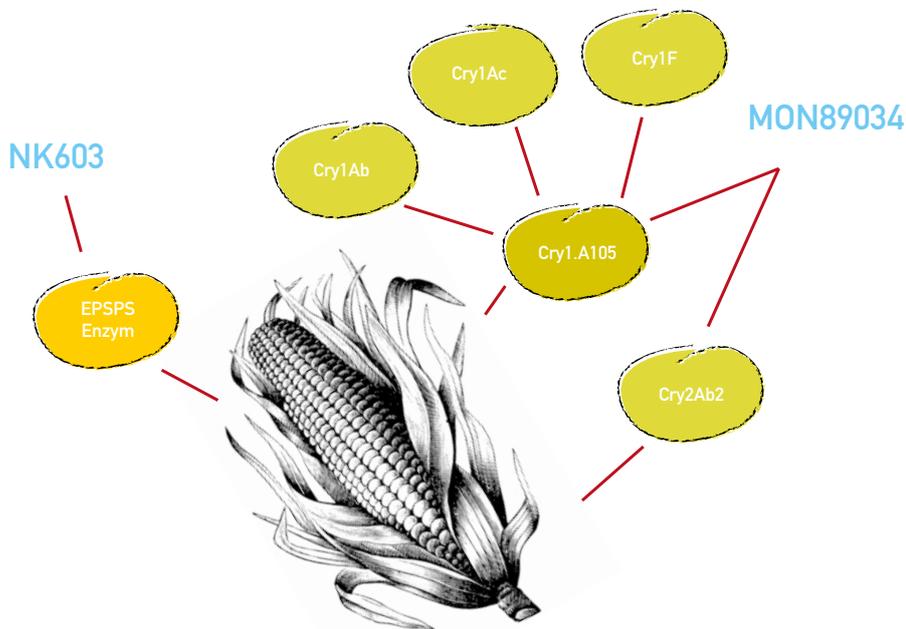


TEST BIOTECH

Testbiotech
Institute for Independent
Impact Assessment in
Biotechnology



Analysis of the data submitted by Monsanto to the Indian authorities on genetically engineered maize **MON89034 x NK603**

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on genetically engineered maize MON89034 x NK603**

Dr. Christoph Then for Testbiotech, January 2013

Imprint

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Summary

Greenpeace India asked Testbiotech to assess data presented by the US company Monsanto to the Indian authorities, for biosafety tests prior to commercial approval of the genetically engineered maize (corn) MON89034xNK603. The said information was obtained through RTI procedure from the Department of Biotechnology and Genetic Engineering Approval Committee (GEAC). This is a stacked event, a combination of genetically engineered plants cross-bred with each other. It produces two insecticidal proteins, one a technically modified version of an existing toxin (C_y2Ab₂) and one, which does not occur in nature, produced from synthetic DNA (C_r1A.105). The plants are also herbicide tolerant (glyphosate).

Nearly all the data presented by Monsanto relate to the parental plants (NK603 and MON89034) and not the stacked plants. Monsanto also conducted most of the investigations themselves or with the involvement of their own members of staff. Independent studies are a rare exception.

The data presented so far is on issues such as molecular characterisation, compositional analysis, agronomical performance, genetic stability, expression of the DNA construct, allergenicity, toxicity, nutritional effects and environmental risks. Closer analysis reveals several flaws:

- › The molecular data are incomplete and partially outdated.
- › More recent technologies such as screening of the activity of the plant's endogenous genes were not applied
- › The data on the expression of the additional DNA shows a high level of variation. No fully evaluated method was made available for determining the content of newly expressed proteins
- › The compositional analysis showed several significant findings that were not investigated further.
- › The pepsin digestion assay used for the assessment of allergenic risks does not provide reliable data; publications showing immune reaction after exposure to genetically engineered plants were not mentioned by the applicant
- › Only proteins produced by bacteria (and not those produced in the plants) were used for acute toxicity tests
- › The proteins were not tested systematically to investigate synergistic or combinatorial effects
- › Feeding studies to investigate health effects were only performed with the parental plants and only for 90 days; the outcome of these investigations is controversial amongst experts
- › Nutritional studies were not conducted with animal breeds used in Indian agriculture
- › No detailed assessment was performed on the residues from spraying with relevant formulations of glyphosate
- › No systematic investigations of potential synergistic or combinatorial effects of the plants constituents were performed
- › Persistence and invasiveness may possibly become a serious issue in India since several plants are known to be compatible with maize plants and could cross breed. Detailed investigations to exclude uncontrolled spread of the transgene into the environment were not performed
- › Although the toxins expressed in the plants do not occur in nature, no detailed investigations

were performed to exclude risks for non-target organisms, human health and Indian biodiversity

- › Recent data on the implications of large-scale applications of glyphosate, such as the emergence of herbicide resistant weeds and biodiversity hazards are completely missing
- › There was almost no data made available on the stacked events, most of the data is on the parental plants.

To conclude, based on the data presented by Monsanto, no decisions can be taken on the safety of the plants in regard to open field trials or commercial cultivation. Apart from missing data and inadequate investigations, there are in fact substantial indications for health and environmental risks. Several publications have revealed effects on the immune system, health risks caused by spraying with the complementary herbicide, unexpected toxicity of the synthetic toxin produced in the plants and long-term effects such as losses in biodiversity as well as increasing resistance in weeds and pest insects.

Introduction

Greenpeace India forwarded several documents to Testbiotech of a stacked genetically engineered maize (corn) plant known as MON89034xNK603. These were obtained under the Right to Information with Act from Department of Biotechnology and Genetic Engineering Approval Committee (GEAC), which is the nodal agency for approval of any environmental release of GMOs in India.

MON89034 produces two insecticidal proteins effective against a broad range of *Lepidoptera* species and NK603 is tolerant to the herbicide glyphosate, known under brand names such as Roundup (which is a brand of Monsanto).

Testbiotech assessed the documents for indications of health effects, environmental risks, reliability and conclusiveness. The data were also compared with data forwarded to the EU authorities, whilst additionally taking into account data from the risk assessment performed by the European Food Safety Authority (EFSA) (EFSA, 2008, EFSA 2009a and EFSA 2009b) and some recent publications.

1. Technical description

Maize MON89034xNK603 was created by crossing two genetically engineered plants, NK603 and MON89034. Several hybrid varieties were produced based on this cross-breeding.

Maize MON89034 was transformed by *Agrobacterium tumefaciens*-mediated gene transfer. Maize MON89034 contains two insecticidal toxins, CryIA.105 and Cry2Ab2:

- CryIA.105 protein is a chimeric, synthetic toxin. It has some similarities with three different toxins originating from *Bacillus thuringiensis* (CryIAc, CryIF, CryIAb), but does not have a true homology in nature.
- Cry2Ab2 is a modified variant of the wild-type Cry2Ab2 protein isolated from *Bacillus thuringiensis*. The CryIA.105 protein is driven by a promoter from the cauliflower mosaic virus (CaMV) 35S RNA with a duplicated enhancer region. The expression of the Cry2Ab2 protein is under control of the 35S promoter from the figwort mosaic virus and contains further parts of foreign DNA. The DNA constructs also contains DNA from wheat and rice.

Maize NK603 was produced by particle bombardment and contains the DNA for expressing two versions of the EPSPS enzyme (CP4 EPSPS and CP4 EPSPS L214P). The DNA is under control of a 35S CaMV cauliflower mosaic virus promoter and a rice actin promoter. The nos-terminator is used to stop gene activity at the downstream end of the DNA construct.

2. Overview of the data submitted

Greenpeace India provided Testbiotech with several documents. The list of studies below, are part of the dossier that was forwarded by the US company Monsanto, to the Indian authorities for the risk assessment of genetically engineered maize MON89034xNK603. The documents were made available on the Indian government's website www.igmoris.nic.in. Some further documents were accessed via the Right to Information law in India.

2.1 MON89034

The list of relevant documents submitted by Monsanto to the Indian authorities can be summarised as follows:

- One unpublished study on molecular characterisation: Rice *et al.*, 2006
- One unpublished study on compositional analysis of the plants: Reynolds *et al.*, 2006
- Four unpublished studies on allergenic risks and / or toxicological risks (database comparison): McClain & Knupp, 2008 (CryIA.105); Knupp & McClain 2008 (Cry2Ab2); McClain & Silvanovich, 2006a (CryIA.105); McClain & Silvanovich, 2006b (Cry2Ab2)
- Two unpublished studies on protein digestibility: Kapadia & Rice, 2005 (CryIA.105) and Kapadia & Rice, 2006 (Cry2Ab2)
- One unpublished study on heat stability: Goley & Thorp, 2005
- One published study on compositional analysis of the plants: Drury *et al.*, 2008
- A report originally presented by Monsanto to the US authorities (compiled by Gao, 2006) with data on molecular characteristics, genetic stability, expression levels of the additional enzyme, phe-

notypic, agronomic, ecological interactions, compositional analysis, acute toxicity of the toxins, environmental impact and agronomic practices

- An application for field trials in India (Monsanto, 2008 and Monsanto, 2011a). Testbiotech is not aware of any published results. This application for field trials with MON89034x NK603 (Monsanto 2008) also provides some expression data for the Bt toxins in MON89034 and a preliminary efficacy study on maize stem borer (*Chilo partellus*)

2.2 NK603

The list of relevant documents forwarded by Monsanto to the Indian authorities can be summarised as follows:

- Two unpublished studies on molecular characterisation: Deng *et al.*, 1999; Astwood *et al.*, 2001
- One unpublished study on genetic stability: Hillyard *et al.*, 2000
- One unpublished study on compositional analysis of the plants and expression of the transferred DNA construct in the plants: Sidhu & Ledesma, 2002
- Three unpublished studies on allergenic risks (database comparison and digestibility of the enzyme): McCoy *et al.*, 2002; McLain & Silvanovich 2007; Leach *et al.*, 2002
- Three unpublished studies on toxicity (database comparison of the EPSPS enzyme with known toxins and the heat stability of the protein): McCoy *et al.*, 2002; McCoy & Silvanovich, 2003; Holleschak *et al.*, 2002
- One published study on molecular characterisation and genetic stability: Heck *et al.*, 2005
- One published study on compositional analysis of the plants: Ridley *et al.*, 2002
- Two published publications on agronomic performance: Thomas *et al.*, 2004 and Heck *et al.*, 2005
- Two published studies deal with feeding studies to investigate toxicity: Harrison *et al.*, 1996; Hammond *et al.*, 2004
- Five published nutritional feeding studies (including rats, cattle and poultry): Chrenkova *et al.*, 2002; Ipharraguerre *et al.*, 2003; Erickson *et al.*, 2003; Taylor *et al.*, 2003; Grant *et al.*, 2003
- One published study on identification of the plants by PCR test: Nielsen *et al.*, 2004
- A report originally presented by Monsanto to the US authorities (Croon *et al.*, 2000) with data on molecular characteristics, genetic stability, expression levels of the additional enzyme, data on pest infestation, agronomic performance and weed resistance
- An application for field trials in India including an experimental protocol for the field trials (Monsanto 2011b). Testbiotech is not aware of any published results.

2.3 MON89034xNK603

Out of all the documents forwarded by Monsanto to the Indian government only one was made available to Testbiotech on the stacked event; one nutritional feeding study on poultry with MON89034xNK603: Taylor *et al.*, 2007.

3. A brief analysis of general data

Below is an analysis of the documents on molecular characterisation, genetic stability, expression of the gene construct, compositional analysis and agronomic performance.

3.1 MON89034

Molecular characterisation

Monsanto compiled and presented the data (Rice *et al.*, 2006; Gao, 2006), none of which were submitted to any external quality control.

The data as submitted shows some unintended changes in the inserted DNA construct. There is a change in the structure of the promotor stemming from the cauliflower mosaic virus. This promotor derived from a virus is known to overlap with parts of a DNA, which codes for a viral protein can render various unintended effects in the plants (Podevin, N. & du Jardin P., 2012). These recent findings require a detailed comparison of the relevant data.

Newer technologies to investigate the transcriptome, the proteome and the metabolome were not applied, so that important information at the molecular level and on the impact of the transgene on the activity of the plant's endogenous genes is missing.

Expression of the transferred DNA construct

Expression data from field trials in the US (Gao, 2006) and from greenhouse trials in India (Monsanto 2008) were submitted to the Indian authorities. This data was not subjected to independent quality control. In particular, the data generated in India are of poor quality, there is no raw data and no indication of the true range of variability; none of the data presented are on expression rates in the grain; there is only a low number of samples; none of the responsible researchers are named etc.

There are, moreover, huge differences between the data from India and that from the US. For example, in the US the mean value for CryIA₁₀₅ in leaf is given as 72-520 µg/g (dry weight) whilst the data from India indicates 22-164 µg/g (dry weight). These differences could be explained by greenhouse conditions in India. However, data from field trials in Argentina (Hartman *et al.*, 2006) that were (as far as we know) not forwarded to the Indian authorities, show substantial differences compared to both the US and Indian data. Data on plant tissue such as leaf are relevant for the usage of the crop as animal feed.

The level of the toxin in the grain is much lower than in the leaf, but data from the US and Argentina also show substantial differences for Bt content in grain. For example, the range of data on CryIA₁₀₅ from the US (4,7-7,0 µg/g, dry weight) and Argentina (1,9-3,2 µg/g, dry weight) do not even overlap. As already stated, there is no data on Bt content in the grain produced in India.

Table: Comparison of Cry1A.105 and Cry2Ab2 expression data from MON89034 in overseason leaf (OSL) from trials in the US and Argentina and in whorl leaf from trials in India dry weight, µg/g. Source: Data from Monsanto (Gao, 2006; Hartmann et al., 2006; Monsanto 2008).

	U.S.A 2005		Argentina 2004		India 2008	
	Cry1A.105	Cry-2Ab2	Cry1A.105	Cry2Ab2	Cry1A.105	Cry2Ab2
Range	27-850	48-270	60-350	64-470	?	?
Mean	72-520	130-180	160-260	120-270	22-164	51-360
Max Bt content per varieties tested (range of data)	1120		820		??	

Altogether, there is a great deal of uncertainty about the true range of expression levels of the Bt proteins in the plants. Several more investigations are needed to gather sufficient data on the expression levels in different varieties and under various environmental conditions, since this is known to impact the Bt content in the plants (Then & Lorch, 2008).

Furthermore, there were no evaluation trials involving several laboratories to establish reliable and comparable protocols for measuring the content of the Bt proteins in the plants. The evaluation of methods is necessary to produce reliable results since even small changes in the protocol in the ELISA tests can produce very different results (Szecaks *et al.*, 2012).

Without reliable data on the expression levels, no conclusions can be drawn on the safety of the products derived from the plants.

Compositional analysis

Monsanto conducted the studies on compositional analysis (Gao, 2006; Reynolds *et al.*, 2006; Drury *et al.*, 2008). The investigations show a broad range of significant differences between the genetically engineered plants and the isogenic lines (comparators). However, none of these differences were investigated in detail. Instead, “historical data” i.e. data from various sources not linked to the actual field trials were used for further comparison. This only served to introduce statistical noise into the data making it impossible to draw proper conclusions on the safety of MON89034. The ILSI (International Life Sciences Institute) database, known to be unreliable, was used for comparison with historical data. As John Perry, Chair of the GMO Panel at the European Food Safety Authority (EFSA), explained in a public hearing¹:

“I think we’re in a situation where we would be unwise at the present time (maybe in the future this will be different), but at the present time we can’t trust the ILSI database. There is not sufficient environmen-

¹ Observations of Mr. Joseph Perry, Vice-Chair, at EFSA’s consultative workshop on its draft guidance for the selection of Genetically Modified (GM) plant comparators, held in Brussels on 31 March 2011, <http://www.efsa.europa.eu/en/events/event/gmo110331.htm>

tal information from where these trials were done and that's why we insist that the commercial reference variety should be planted simultaneously with the GM and the non-GM. Otherwise I think we are in an unsafe situation and I would worry that the limits would be too wide."

Consequently, all comparisons to these historical data should be excluded from the risk assessment. Further, Monsanto uses phrases such as "biological relevance" which in themselves indicate some limits in the interpretation of significant differences in the data. As a result, a lot of uncertainties remain regarding the compositional analysis of MON89034.

It is known from several publications that genetically engineered plants can react differently to plants derived from conventional breeding (see for example Meyer *et al.*, 1992; Gertz *et al.*, 1999; Matthews *et al.*, 2005; Zeller *et al.*, 2010). Maize growing regions in India such as Uttar Pradesh, which is semi-humid to arid², and Karnataka, which is semi-arid to dry sub-humid³ have a broad range of extreme climatic conditions that might become even more extreme with ongoing climate change.

A test with MON89034 using controlled conditions in climate chambers including exposure to various biotic or abiotic stressors should be the starting point to find out in more detail about the plants' reactions under changing environmental conditions with regard to compositional analysis, agronomic performance, expression of the Bt proteins and genetic stability.

Agronomic performance

Gao (2006) includes some data on agronomical performance such as plant growth and development, yield, plant and ear morphology, plant health and pest susceptibility. Monsanto conducted the investigations under US agricultural conditions.

The data show several significant differences between the MON89034 and its comparators. However, these differences were not investigated in detail. Instead, the investigators used "historical data" that have nothing to do with the MON89034 maize. This once more only served to introduce statistical noise into the interpretation of the data, thereby preventing proper conclusions from being drawn on the safety and comparability of MON89034 with its comparators.

Much more investigation is necessary to assess the impact of environmental factors on the plant's components, and to find out what differences actually exist and how they should be interpreted. For this purpose, investigations should be conducted under contained and defined environmental conditions including various biotic or abiotic stressors. In open field trials it is hardly possible to identify the real impact of the various environmental conditions without having some reliable data from a 'stress test' under defined conditions already. Thus a proper "step by step" procedure should be applied.

2 <http://dacnet.nic.in/farmer/new/dac/AgroClimaticZones.asp?SCod=20>

3 <http://dacnet.nic.in/farmer/new/dac/AgroClimaticZones.asp?SCod=08>

3.2 NK603

Molecular characterisation

Some of the data submitted show unintended changes in the inserted DNA construct. Unintended side effects at the molecular level are concomitant with particle bombardment (ballistic acceleration of particles carrying the additional DNA) Such technical flaws should be a reason to request a set of updated data, using appropriate technologies to investigate the transcriptome, the proteome and the metabolome.

The data as submitted are outdated. None of the studies presented in this context (Deng *et al.*, 1999; Croon *et al.*, 2000; Astwood *et al.*, 2001; Heck *et al.*, 2005) were conducted independently of Monsanto. Only Heck *et al.* (2005) is a fully published paper. Deng *et al.* (1999), Croon *et al.* (2000) and Astwood *et al.*, (2001) were not subject to any external quality control.

As more recent research shows, the nos-terminator meant to stop the biological activity of the inserted DNA construct does not work properly. More detailed analysis shows that a fusion RNA is produced (read through transcript) from the inserted DNA and the neighbouring endogene DNA of the plant. As EFSA says, 2009:

“(...) the data did demonstrate that an RNA species could be detected that likely initiated in the promoter of the NK603 insert and proceeded through the nos 3’ transcriptional termination sequence continuing into the maize genomic DNA flanking the 3’ end of the insert.”

EFSA assumes that only very low levels of the unintended RNA is produced and that the polypeptides being produced are unlikely to be toxic or allergenic. However, Zhang *et al.* (2011) showed that via consumption short pieces of RNA can be transferred from the plant to mammalian cells and can interact with the cell regulation. Thus, risk assessment of NK603 should also include this issue. There should also be a request for independent studies investigating the transcriptome, the proteome and the metabolome.

In conclusion, the data submitted are not independent, not comprehensive and outdated. The plants suffer from technical deficiencies such as unintended changes in the structure of the DNA, a dysfunctional nos-terminator and unintended readthrough RNA. Furthermore, the promotor derived from cauliflower mosaic virus is known to overlap with parts of a DNA which codes for a viral protein that can render various unintended effects in the plants (Podevin, N. & du Jardin P., 2012). These recent findings require a detailed comparison of the relevant data.

Expression of the transferred DNA construct

Sidhu & Ledesma (2002) and Croon *et al.* (2000) both present identical data from field trials in the US conducted in 1998. The results show a content around 25 µg/g fresh weight (fw) in forage and 11 µg/g fw in kernels. Monsanto carried out these studies but there were no independent measurements to confirm a certain range of DNA expression. No data on dry weight were given, which are used in many other trials, so that the data cannot be compared easily.

Furthermore, no testing was carried out with the involvement of other laboratories – which would be necessary to establish reliable and reproducible protocols for measuring the content of the EPSPS enzymes as produced in the plants. Such evaluated testing methods are indispensable for generating reliable results since it is known that small changes in protocol when carrying out ELISA tests can render highly different results.

Furthermore, climatic conditions or varietal effects in the plants can play a significant role in the expression of the DNA constructs. As the data show, a much higher content of the EPSPS enzyme were reported in other trials. For example, data reported by Heck *et al.*, 2005, show a much broader variation than reported by Sidhu & Ledesma (2002). Also data forwarded to EFSA (2009) differs substantially from that of Sidhu & Ledesma (2002):

“In maize forage, the mean CP4 EPSPS protein levels from the four different field sites in Europe were as follows: 44.2 µg/g fw (fresh weight) (site 1, Southern France), 45.7 µg/g fw (site 2, Southern France), 43.6 µg/g fw (site 3, Northern France), and 60.9 µg/g fw (site 4, Italy). The overall mean CP4 EPSPS protein level in maize forage across all four sites was 48.6 µg/g fw. In maize grain, the respective values for the 4 sites were 13.2 µg/g fw, 12.7 µg/g fw, 2.2 µg/g fw and 5.5 µg/g fw. The overall mean CP4 EPSPS protein level in maize grain across all four sites was 8.4 µg/g fw.”

Much more investigation is necessary to assess the impact of environmental factors on the expression of the additional gene construct. For this purpose, investigations should be conducted under contained and defined environmental conditions including various biotic or abiotic stressors.

Compositional analysis

All the studies on compositional analysis (Sidhu & Ledesma, 2002; Ridley *et al.*, 2002; Croon *et al.*, 2000) were conducted with the involvement of Monsanto. Only one study was published (Ridley *et al.*, 2002), and the Sidhu & Ledesma (2002) study is not even fully in line with GLP (Good Laboratory Practice) standards as required in the US.

The investigations show a broad range of significant differences between the genetically engineered plants and the isogenic lines. However, none of these differences were investigated in detail. Instead, “historical data” that have nothing to do with the NK603 maize, were applied. This mainly served to introduce statistical noise and prevent any proper conclusions from being drawn on the safety of NK603.

References to interpretations such as “biological relevance” indicate real limitations in the interpretation of significant differences in the data. Thus, there is still a lot of uncertainty in the compositional analysis of NK603.

Many more investigations are necessary to understand the impact of environmental factors on the plant’s components and to find out what differences actually exist and how they should be interpreted (see points under MON89034). For this purpose, it is essential to carry out investigations under

contained and defined environmental conditions including various biotic or abiotic stressors (similar like a 'stress test'). Further, there should be a comparison between the sprayed and the non-sprayed genetically engineered plants since the metabolism of the herbicide can also impact plants composition.

Agronomic performance

Croon *et al.*, (2000) Heck *et al.* (2005) report some data on agronomical performance such as plant growth and development, yield, plant and ear morphology, plant health and pest susceptibility. The investigations were conducted with the involvement of Monsanto.

It appears that no raw data from these investigations were submitted to the Indian authorities, so the only source of evaluation of this issue is a table with some results in Heck *et al.*, (2005) and Croon *et al.* (2000). The data are not coherent, since Croon *et al.* (2000) report significant differences between the NK603 and its isogenic line that are not mentioned by Heck *et al.*, 2005. Further, Thomas *et al.*, (2004) which seems to be one of the very rare independent publications on NK603 that were forwarded to the Indian authorities, reports a reduced viability and a reduced amount of pollen if the plants were sprayed with glyphosate. Contrary to these findings Heck *et al.*, (2005) claim that there is no difference in pollen shed.

4. Health risks

Below is an analysis of the documents on allergenicity, toxicity and nutritional quality.

4.1 Mon89034

Allergenicity

Four studies on allergenic risks, performed by staff members of Monsanto, were presented to the Indian authorities. Two on the protein Cry1A.105 (McClain & Knupp, 2008; McClain & Silvanovich, 2006a) and two on the protein Cry2Ab2 (Knupp & McClain 2008; McClain & Silvanovich, 2006b). These studies are based on database comparisons with known allergens that showed no similarity.

However, there was no study to investigate adjuvant effects. There are several proteins in maize that can cause allergic reactions. The newly introduced gene construct might, for example, enhance an immune response in these endogenous allergens of the plants. It is known that bacterial proteins very often elicit immune reactions. Especially Cry1Ac (which shows some similarity to Cry1A.105) is known to enhance immune reactions and able to bind to epithelial cells in the intestine of mice (Vázquez-Padrón *et al.*, 1999, Vázquez-Padrón *et al.*, 2000). Therefore, several more detailed investigations should be conducted on potential immune reactions that could be triggered by the Bt proteins.

This concern was also raised by experts of the Norwegian authorities in the context of the market authorisation of MON89034xNK603 in the EU. (EFSA 2009c):

“Assessment of allergenicity of the whole GM plant or crop Scientific studies, also very recent ones, have shown that the Cry1Ac protein is a potent systemic and mucosal adjuvant, which is an enhancer of immune responses. The GMO Panel of the Norwegian Scientific Committee for Food Safety find it difficult, based on the available data, to assess whether kernels from maize MON89034 may cause more allergenic reactions than food and feed from unmodified kernels. As the different Cry proteins are closely related, and in view of the experimental studies in mice, the GMO Panel finds that the likelihood of an increase in allergenic activity due to Cry1A.105 and Cry2Ab2 protein in food and feed from maize MON89034 cannot be excluded.”

Further, the isolated proteins were subject to digestibility tests in a pepsin digestion assay (Kapadia & Rice, 2005, on Cry1A.105 and Kapadia & Rice, 2006, on Cry2Ab2). As a result, the Cry proteins are thought to be degraded rapidly in the gastrointestinal tract. However, these tests do not allow assessment of digestion of the protein under realistic conditions when mixed with other compounds in food and feed. For example, feeding studies with pigs conducted by Chowdhury *et al.* (2003) as well as Walsh *et al.* (2011) found that Cry1A proteins can frequently be found in the colon of pigs. Thus, the Cry1A proteins can show much higher stability in monogastric species than predicted by current in vitro digestion experiments. These findings underline the need for more detailed investigations on MON89034 and the digestibility of the Cry proteins under realistic conditions.

There are already several studies showing that genetically engineered plants producing Cry proteins interact with the immune system in vertebrates. Such examples include fish (Sagstad *et al.*, 2007), pigs (Walsh *et al.*, 2011), mice (Finamore *et al.*, 2008), and rats (Kroghsbo *et al.*, 2008, Gallagher, 2010). Again, this highlights the need for immunological studies to assess the health risks in detail.

Toxicity

MON89034 produces two different insecticidal toxins. Cry1A.105 has never been used before – it is a newly synthesised toxin that has no variants in nature. Cry2Ab2 has previously been used in Bt cotton, but – as far as we know – not in plants that are mainly used for food and feed production.

Toxicity testing (in relation to human health and the environment) of MON89034 has to follow a specific strategy. There must be investigations not only on the toxicity of the single proteins, but also on the toxicity of the combined toxins.

In general, the mode of action of Bt toxins is not fully understood. It is a controversially debated issue (Pigott & Ellar, 2007; Then, 2010). Strict selectivity of the Bt toxins in most cases is not shown by empirical evidence and more recent research, shows that there are mechanisms that might cause toxicity in other species and even in mammals (Soberon *et al.*, 2009). Thus, risks for human health cannot be excluded by assumptions or considerations but only by empirical testing before market authorisation. Each Cry protein has its own specific way of interacting with the target organism. As Gao (2006) describes it:

“The mechanism of insecticidal activity or mode of action of Cry proteins consists of a number of steps e.g. solubilisation, activation, receptor binding, oligomerisation, and pore formation (...). There are important differences in each step of the mode of action, which influences the interactions of these proteins with susceptible insects without qualitatively influencing their host range. Several lines of evidence establish that Cry1A.105 and Cry2Ab2 have important differences in their mode of actions, particularly in the way in which they bind to the lepidopteran midgut. These proteins have different primary structures, share only 14% of amino acid sequence identity, and bind to distinct proteins in the midgut of target species at different rates with different affinities.”

However, the exact mode of action of the toxins was not studied in detail. Gao (2006) only very vaguely confirms essential differences between the binding mechanisms of the relevant Bt toxins:

“Monsanto’s research data indicates that there are important differences between the Cry1A.105 and Cry2Ab2 proteins regarding their binding to the lepidopteran midgut. The data demonstrates that Cry1A.105 and Cry2Ab2 proteins bind to some unique components on European corn borer brush border membrane. They also share many common binding sites.”

But without understanding how the toxin works in detail, risk assessment is a difficult task.

There are many other open questions concerning the safety of the proteins. Some plant enzymes that diminish the digestion of proteins (protease inhibitors) can strongly enhance the toxicity of Bt toxins (Pardo Lopez *et al.*, 2009). Even the presence of very low levels of protease inhibitors can multiply the insecticidal activity of some Cry toxins. It is known that maize produces such inhibitors (Shulmina *et al.*, 1985).

Synergistic effects can become highly problematic for non-target organisms. Interactivity between the toxins or in combination with environmental toxins, bacteria, plant enzymes or pesticides can cause unexpected higher toxicity and lower selectivity (Then, 2010). These effects can impact human health as well as ecosystems.

As Pardo Lopez *et al.* (2009) and Pigott *et al.* (2008) show, synthetically derived and modified Bt toxins can show much higher toxicity than native proteins. Even small changes in the structure of the proteins can cause huge changes in toxicity. In the case of CryIA.105, there is indeed evidence that toxicity is enhanced and selectivity is decreased. As a result, the toxicity of CryIA.105 can concern a wider range of non-target organisms than is expected. As described in Monsanto's US patent application Patent 6,326,169, Polynucleotide compositions encoding CryIAC/CryIF chimeric O-endotoxins - the toxicity is changed and enhanced in an unexpected way:

“Another aspect of the invention further demonstrates the unexpected result that certain chimeric CryIAb/CryIF proteins maintain not only the insecticidal characteristics of the parent delta -endotoxins, but also exhibit insecticidal activity which is not displayed by either the native CryIAb or CryIF endotoxins.”

These findings on unexpected changes in the toxicity of the Bt protein, which were not forwarded to the Indian authorities, show the need for much more detailed investigation into the potential effects of Bt toxins on other species and not just on insects.

Monsanto did not present a feeding study with different parts of the plant to investigate health effects. Gao (2006) only reports on a short term acute toxicity study with the isolated protein. The proteins were not fed in combination, thus synergistic effects were not investigated.

Monsanto conducted a study on the heat stability of the toxin (Goley & Thorp, 2005). But this study used very high temperatures (forced air electric oven at approximately 204°C for 20 minutes to simulate heat processing used in the commercial processing of corn grain). There are many other ways to process maize including temperature treatments, hydrolyses, soaking in slightly acidic water, and drying, all of which were not taken into account. In India, it is common for corn flour to be used for making flat breads (rotis); roasting over charcoal is also common. It is not clear, to which extent consumers and animals will be exposed to the Bt proteins through food and feed.

It should be noted that a 90-day-sub-chronic feeding study with the plant was conducted and presented to the European Food Safety Authority (EFSA). EFSA has accepted this feeding study believing it to show no health risks. However, experts from the authorities of some European Member States have raised concerns about significant findings. Female animals in particular showed several complications in their kidneys. Experts from the Belgian authorities describe it thus:

“The kidneys of the high-dose (33%) test group females showed findings not found or at lower incidence in the control group. One rat was found dead on day 14. There were 5 findings of chronic progressive nephropathy, 3 findings of transitional cell hyperplasia, 2 cases of sub-acute inflammation and hydronephrosis, papillary necrosis and tubular necrosis. Most of these findings were attributable to the two rats which were found to have calculi (see Macroscopic examination). It is worth discussing these items and have a closer look, whether these findings are solely due to chance. In case any doubt remains, further testing is recommended.”

To date, no other sub-chronic feeding study has been presented and no results from any chronic feeding studies published. Therefore, no final conclusion can be drawn on the safety of the plants.

Nutritional studies

Gao (2006) mentioned two studies with avian species, however, no details are given on test protocol and raw data.

None of these studies showed cause for concern. However, even if conducted independently and accurately, nutritional studies cannot be used to draw conclusions on health risks because the endpoints measured are mostly of economical relevance (such as meat or milk production over short period of time) and are not appropriate for identifying potential health impacts in humans.

Further, it should be mentioned that no study was conducted with animal breeds used in Indian agriculture. Thus the studies submitted are of only minor relevance, and not sufficient for a comprehensive risk assessment.

4.2 NK603

Allergenicity

Three studies on allergenic risks, performed by Monsanto's own members of staff, were presented to the Indian authorities (McCoy *et al.*, 2002; Leach *et al.*, 2002; McLain & Silvanovich, 2007). These studies are based on database comparisons with known allergens that showed no similarity (McCoy *et al.*, 2002; McLain & Silvanovich *et al.*, 2007). The results of these studies is in accordance with other investigations concerning this enzyme.

However, there was no study carried out to investigate adjuvant effects. There are several proteins in maize that can cause allergic reactions. The newly introduced gene construct might, for example, enhance an immune response to these endogenous plant proteins. It is known that bacterial proteins very often elicit immune reactions. Therefore, more detailed investigations should be conducted.

The protein was subject to digestibility tests, which show that the isolated protein is digested rapidly (Leach *et al.*, 2002). However, these tests do not allow the assessment of digestion under realistic conditions when the protein is mixed with other compounds in food and feed. Thus, for example, feeding studies with pigs should be conducted to find out more about what happens to the protein in the intestine.

Toxicity

Four studies produced by Monsanto are on the toxicity of the isolated EPSPS enzyme: McCoy *et al.*, 2002; McCoy & Silvanovich, 2003; Holleschak *et al.*, 2002 and Harrison *et al.*, 1996. According to these studies, the enzyme does not show any acute toxicity, which is in accordance with other findings and publications.

However, the applied methodology using the enzymes produced by bacteria as opposed to enzymes isolated from the plants is a cause for concern since there might be small differences in the structure of the protein that might be missed in the investigations.

The heat stability of the protein was assessed by heating maize to a temperature of 204°C for 15 or 30 minutes, which is a very high temperature for maize processing. There are other methods of maize processing that were not taken into account (details see above for MON89034).

A particular matter of concern is that no data were submitted on the residues from spraying with the herbicide formulations and their metabolites in the plant. To produce relevant data, various concentrations and formulations of the herbicide should be used to determine the actual range of residues.

There are several reasons why the risk assessment of genetically engineered plants with herbicide tolerance cannot leave aside the issue of residues from spraying. Several experts are warning that a higher toxicity has to be expected (for example Benachour *et al.*, 2007; Paganelli *et al.*, 2010). In this context, the additive POEA (polyethoxylated tallow amine) must be considered as it is even more toxic than glyphosate in the plants. In 2010, German authorities even prohibited the usage of certain glyphosate formulations with a high content of POEA for the production of animal feeds in order to avoid the risk of passing toxins into the food chain⁴.

This problem is also relevant in the context of the 90-day feeding study that was conducted with the involvement of Monsanto (Hammond *et al.*, 2004). In this study, which showed a broad range of significant findings that were not investigated further (see Spiroux de Vendômois *et al.*, 2009), the actual glyphosate formulation and its concentration was not determined.

Finally, no long-term chronic feeding studies were submitted to the Indian authorities. Very recently, Seralini *et al.*, 2012 found signals for toxicity and carcinogenicity when rats were fed with NK603 and low dosages of glyphosate formulations. While the outcome of this study is controversial, one should bear in mind that other relevant studies are mostly missing. For example, the German Federal Institute for Risk Assessment (BfR) states that Seralini *et al.* (2012) is the only feeding study so far to examine the long-term effects of a herbicide formulation of glyphosate (in this case, Roundup):

“The BfR has noticed with interest that for the first time a chronic feeding study with glyphosate mixture was conducted. So far there are no such chronic studies available, because the regulations in place globally only request toxicology studies with the active ingredient.” (Unofficial translation).

As a consequence, the Indian authorities should request further independent long-term feeding studies before any decision is taken on the marketing authorisation for NK603.

Nutritional studies

Monsanto presented five nutritional studies (including rats, cattle and poultry). Four of them were conducted involving Monsanto members of staff. (Ipharraguerre *et al.*, 2003; Erickson *et al.*, 2003; Taylor *et al.*, 2003; Grant *et al.*, 2003). The only study that might be seen as independent (Chrenkova *et al.*, 2002) does not follow a common test protocol.

None of these studies found cause for concern. However, even if conducted independently and accurately, nutritional studies cannot be used to draw conclusions on health effects because the endpoints measured are mostly of economical relevance (such as meat or milk production over short period of time) and are not appropriate for identifying potential impacts on human health.

Further, it should be mentioned that no study was conducted with animal breeds used in Indian agriculture. So the studies as submitted are only of minor relevance and inadequate for a comprehensive risk assessment in India.

4 www.bvl.bund.de/DE/04_Pflanzenschutzmittel/05_Fachmeldungen/2010/psm_anwendungsbestimmungen_tallovamin-Mittel.html

4.3 MON89034 x NK603

Only one nutritional feeding study on poultry with MON89034xNK603 was forwarded to the Indian authorities. This study was conducted by Monsanto (Taylor *et al.*, 2007). In the light of the high level of uncertainties regarding the safety of the parental plants, this lack of any meaningful investigation into effects on health of the stacked events is not acceptable.

Consideration should be given to potential new combinatorial effects in the stacked plants that give cause for further concerns about safety. For example, combinatorial effects might be caused by residues in the plants from spraying with the insecticidal proteins.

In this context, it is interesting to look at the risk assessment performed in the EU. In the US no specific risk assessment is performed on stacked plants, but the EU requests a separate application for the stacked events and does not only assess the parental plants. Due to this difference in regulations, the EU is the only region so far where MON89034xNK603 has been subjected to any risk assessment.

The European Food Safety Authority (EFSA) is being criticised from various sides to perform only a superficial risk assessment. In this case, ten EU member states raised concerns: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Italy, Spain, The Netherlands and also Norway (EFSA 2009c).

Amongst others, the following issues were raised:

- › The artificial toxin CryIA.105 should have been investigated in much more detail;
- › The potential synergistic effects of the toxins were not fully investigated;
- › No animal feeding studies to investigate potential effects on health were performed with the stacked event;
- › No long-term feeding studies were performed;
- › The results from the feeding studies with the parental plants should be investigated further;
- › Specific allergenic risks caused by were overlooked;
- › Significant differences in the composition of the plants should be investigated further.

For example, experts from Germany gave the following comments:

“it has to be noted that CryIA.105 is a synthetic protein, which had never been used in commercial microbial insecticides or other transgenic plants before, while experience with Cry2Ab2 is also limited and combinatory effects of both toxins were hardly studied.”

“Because indications for possible adverse effects of MON89034 maize on mammals (urinary calculi, significantly different nephrological and haematological findings) were observed in studies provided with application EFSA/GMO/NL/2007/37 for authorisation of MON 89034 maize (...), the applicant is asked to provide a feeding study with the whole food and feed, i.e. MON89034 x NK603 maize compared with maize with a comparable genetic background, in mammals. We suggest conducting at least a 90-day feeding study addressing, in particular, haematology and nephropathology.”

Despite concerns raised by several Member States, the EU has accepted the import of the product and its usage in food and feed (but not for cultivation). This decision should be viewed with great caution and in no way be a precedent for market authorisation in India. The EU has a system of labeling and segregation in place. Currently all the larger food producers and retailers avoid using ingredients from genetically engineered plants in their products which means that consumers do not normally come into contact with them. If it is imported it will only be used for animal feed (or non-food purposes). In India, systems such as segregation, are not fully established. The range of products that could be used in food and feed encompasses various parts of the plants, harvested at different stages of cultivation and processed in many ways.

5. Environmental risks

Only minimal data were made available on environmental risks. Some data on environmental risks posed by glyphosate and Bt toxin were partially outdated and did not have anything to do with the ecosystem in the various geo-climatic regions in India.

5.1 Persistence and invasiveness

As Monsanto (2011) describes in its application for field trials in India, there are many wild plant species in India that are sexually compatible with maize:

“The original genera of Maydae viz. Coix Polytoca, Sclerachne, Trilobachne and Choinachne are all native to the region extending from India and Burma through the south East Asia into Australia. America genera are not native to this region. Out of the oriental genera Choinachne species are widely distributed in India, particularly in the dry region of the eastern and western Ghat, Tamil Nadu and other parts of the country.

Some species prefer marshy lands and are notices widely in Madhya Pradesh, Tamil Nadu, Maharashtra, Andhra Pradesh, Karnataka, Gujarat and Himachal Pradesh. Polytoca and Trilobachne are found in eastern parts of the country as well as in eastern and Western Ghats. Mostly all these wild relatives of maize are found away from agricultural land in forests and hilly tracks. However, in some cases eg. In Andhra Pradesh Coix are found to grow on river banks, irrigation channels and other places near to agricultural lands.

Although inter-generic crosses involving maize and other grasses are possible under controlled conditions, some genetic barriers seem to naturally exist in reducing free hybridization. Further, occurrence of Teosinte is extremely rare.”

Wild relatives would be of great relevance if any large-scale field trials or even and commercial cultivation were to be allowed. Detailed experiments are indispensable to assess the risk of DNA constructs from the genetically engineered maize escaping from the fields into the wild species. These must encompass all relevant species to investigate, for example, heterosis effects that can lead to persistence and invasiveness of the emerging hybrids. Such data – that should be produced in the green house and not in open field trials - are necessary draw conclusions on the long-term effects of commercial cultivation and the possibility of controlling or even withdrawing the maize from the market in the event of unexpected risks and hazards.

5.2 Bt plants, environmental risks and the impact on agriculture

In the case of MON89034 and its specific Bt proteins, the exact mode of action was not investigated, even though this would be highly relevant for assessing the toxicity. Each Cry protein seems to have its own specific way of interacting with the target organism. But without understanding how the toxin works, risk assessment is a difficult task.

As mentioned, in the case of CryIA.105, there is evidence that toxicity is enhanced and selectivity is decreased, the toxicity is changed and enhanced in an unexpected way.

Gao (2006) summarises the risks for non-target organisms such as collembola, earthworm, honeybee larvae, minute pirate bug, ladybird beetle and parasitic wasp. However, no raw data were submitted and there was no detailed protocol on how the testing was done. Without a detailed test protocol, no judgement can be made on the validity of the data presented.

Besides this, several aspects of the risk assessment for non-target organisms are flawed:

- The dosage applied did not take into account the full range of Bt content presented in the expression data. For example to test leaf material, a dosage of 240 µg of Cry1A.105 and a dosage of 210 µg of Cry2Ab2 was chosen, which is not in line with the highest expression rates as described (see above).
- No combined testing of the proteins was conducted in non-target organisms.
- No non-target species or endangered species that are typical and relevant for India were included in the investigations.

In conclusion, no reliable assessment can be made of risks for the environment based on the data presented.

Furthermore, as mentioned, there was no testing carried out with the involvement of independent laboratories to establish a reliable and reproducible protocol for measuring the content of the two Bt proteins produced in the plants. Without reliable data on the expression level, no conclusions can be drawn on environmental risk or the efficacy of the plants in killing pest insects. These data are also needed to assess the risks of emerging resistance in pest insects. The need for these data is underlined by another Monsanto (2008) report showing that MON89034 can be the cause not only mortality but also of non-lethal effects such as retardation in growth and development in neonates of *Chilo partellus* which is supposed to be the most destructive pest insect in maize. If some of the pest insects can survive under field conditions, resistant populations can be expected to emerge quickly.

In any case, long-term experience shows that the emergence of secondary pests or resistance in pest insects will require the application of pesticides and/or cultivation of multi-stacked plants. Such technology cannot be considered adequate for sustainable agriculture (Then, 2010).

5.3 Weed resistance and agricultural practice with herbicide applications

Two studies submitted and carried out by Monsanto mention herbicide resistance in weed. Heck *et al.* (2005) is assuming:

“Another feature of this weed management system has been the slow development of resistance in wild plant populations despite glyphosate use for over 28 years. Currently, only three resistant weed biotypes have been identified (...).”

Croon *et al.* (2000) explain:

“Although it cannot be stated that evolution of resistance to glyphosate will not occur, the development of weed resistance to glyphosate is expected to be a very rare event because:

1. *Weeds and crops are inherently not tolerant to glyphosate, and the long history of extensive use of glyphosate has resulted in few instances of resistant weeds;*

2. *Glyphosate has many unique properties, such as its mode of action, chemical structure, limited metabolism in plants, and lack of residual activity in soil, which make the development of resistance unlikely;*
3. *Selection for glyphosate resistance using whole plant and cell/tissue culture techniques was unsuccessful, and would, therefore, be expected to occur rarely in nature under normal field conditions.”*

Apparently, these assumptions and explanations were completely wrong. Meanwhile, at least 23 weed species have been described as glyphosate-resistant (www.weedscience.org). These weeds show a high rate of expansion in the US, where at least 31 States are affected. There is no doubt, that the main reason for the emergence of these herbicide resistant weeds is the large-scale cultivation of herbicide resistant plants.

Reports show a strong increase in herbicide applications in these crops (Benbrook, 2012), with a high impact on the environment. For example, the large-scale cultivation of glyphosate tolerant plants has affected the biodiversity of plants, which the larvae of the Monarch butterfly feed on. This is likely to have caused a heavy reduction in Monarch populations. Brower *et al.* (2011) and Pleasants & Oberhauser (2012) have shown that in the US and Mexico, a reduction in milkweed species leads to a dramatic decline in the population of Monarch butterflies.

Further there is substantial indication that plant diseases, e.g. increased infestation with fungal diseases (Johal & Huber, 2009), are caused by the large-scale cultivation of glyphosate tolerant crops. The negative impact on plant growth and plant health can even be transmitted to other plants cultivated in the same field in the following year (Bott *et al.*, 2008).

Even EFSA (2009b) found that adverse environmental effects can be caused by the application of glyphosate in crop cultivation:

“These potential adverse environmental effects comprise (1) the evolution of less desirable weed assemblages leading to reductions in farmland biodiversity; (2) the evolution of weed resistance; and (3) effects on soil microbial communities. The magnitude of these potential adverse environmental effects will depend on the specific herbicide management applied at the farm level.”

It should also not be overlooked, that there are substantial risks for rural communities and farmers in those regions with extensive glyphosate applications. In fact, the negative effects of the cultivation of glyphosate-tolerant crops actually affect rural areas as a whole, rather than just agriculture. For example, this can be shown by experience from the US: A study conducted in Mississippi and Iowa in 2007 and 2008 showed that glyphosate was present in most of the samples of air and rainwater taken (Chang *et al.*, 2011). Battaglin *et al.* (2011) identified glyphosate in 93 percent of all soils samples analysed, 70 percent of rainwater samples, 50 percent of smaller rivers and 20 percent of the lakes. Herbicide drift from the fields might be detrimental to the yield in all regions where those plants might be grown, especially where agriculture is on a small-scale and fields are small.

A number of studies have established a link between glyphosate application and illnesses developed by farmers (PAN, 2009). Laboratory tests on amphibians and avian embryos led Paganelli *et al.* (2010) to warn of the human health risks of this phenomenon.

6. Discussion and conclusions

Some of the issues presented and discussed are summarised in Table 2. Some recommendations for further investigation of MON89034xNK603 are given in the overview of this table.

Table 2: Overview of data presented to the Indian authorities for risk assessment of the stacked event MON89034xNK603 and some recommendations for further risk assessment.

Issue	Parental plants (PP) or stacked plants (SP)	Investigated Yes/No	Deficiencies
Molecular characterisation	PP	Yes	No profiling of the transcriptome, proteome and metabolome. No independent studies.
Genetic stability	SP	No	
	PP	Yes	No investigations of functional stability under stress conditions. No independent studies.
Expression of the new proteins	SP	No	
	PP	Yes	The high variability of the data, should be investigated further by using fully established protocols for quantification. No independent studies.
Compositional analysis and agronomical data	PP	Yes	Significant differences should have been investigated further. Plant composition should have been tested under stress conditions. No independent studies.
	SP	No	
Allergenicity of the new proteins produced in the plants	PP	Yes	Pepsin digestion test of isolated protein is not reliable. Digestion of the proteins should have been studied under realistic conditions. Tests with sera from patients that are known to be susceptible to maize proteins should have been carried out. No independent studies.
	SP	No	

Issue	Parental plants (PP) or stacked plants (SP)	Investigated Yes/No	Deficiencies
Other impacts on the immune system	PP	No	Immune reactions were not investigated in detail despite several feeding studies show immune reactions to genetically engineered plants producing bacterial proteins.
	SP	No	
Toxicity of the new proteins produced in the plants	PP	Yes	Only the single proteins produced by bacteria were tested in mice. The toxicity of the proteins as produced in the plants was not tested using more susceptible <i>in vitro</i> methods such as human cells. No independent studies.
	SP	No	
Synergistic or combinatorial impacts of the of the new proteins produced in the plants	PP	No	Health impacts due to the combination of proteins were not investigated in detail, with and without spraying of the relevant glyphosate formulations. Synergistic or combinatorial effects were only tested in some pest insects, but not for other insects or any other non-target organisms.
	SP	No	
Health impacts of plant material	PP	Yes	Only 90 days studies, the results are a matter of controversial discussions Long term feeding studies including several generations were not performed. No independent studies.
	SP	No	
Nutritional effects of the plant material	PP	Yes	Only a few studies were conducted independently from Monsanto. Methods did not follow defined uniform standards. No tests were performed with animal breeds used in India. Only one study with poultry. No independent studies.
	SP	Yes	

Issue	Parental plants (PP) or stacked plants (SP)	Investigated Yes/No	Deficiencies
Invasiveness and persistence	PP and SP	Not investigated in detail	Some general considerations were presented, but hardly based on any experimental investigations Detailed experiments were not performed with all relevant species to investigate, for example, heterosis effects.
Risks to the environment from Bt plants	PP and SP	No relevant data for India.	Data as presented are not conclusive for biodiversity of India. As a first stage, systematic testing in laboratories should be conducted to find out more about the toxicity of the Bt proteins non-relevant non-target species. Interactions with residues from spraying should be taken into account.
Effects of Bt plants on agricultural practice	PP and SP	No relevant data for India	Data on emergence of secondary pests or potential resistance in Indian pest insects are largely missing. But initial data indicate that important pest insects will not be controlled sufficiently by the crops. As a first step, systematic investigation should be conducted on potential pest insects that will not be controlled by the plants and that could even derive advantages from large-scale cultivation.
Risks of cultivation of herbicide-resistant crops	PP and SP	No relevant data for India.	Long term experience from countries such as the US and Argentina show a wide range of negative impacts on the environment that should be fully assessed by Indian authorities.
Impact of cultivation on agricultural practise	PP and SP	No relevant data for India.	Studies provided are completely outdated. Increase in weed resistance and herbicide application have to be expected when these crops are cultivated. Long-term impact on sustainable agriculture should be taken into account in any decision making.

In conclusion, based on the data presented by Monsanto, no decision can be taken on the safety of the plants in regard to large scale field trials and their long-term impact once introduced into the Indian market.

In addition, there are some substantial indications of risks for human health and the environment, which should trigger a high level of precaution in all further decision making:

- Several publications show a reaction in the immune system of animals fed with genetically engineered plants that produce bacterial proteins.

- › There are indications that feeding animals with genetically engineered plants such as NK603 has a negative impact on organs such as the kidneys.
- › Combinatorial effects are likely to occur in Bt-toxins.
- › Health risks due to residues from spraying with glyphosate formulations such as Roundup are a particular cause for concern.
- › Large scale cultivation of the Bt producing plants is likely to result in secondary pests and/or resistance in pest insects.
- › Large scale cultivation of glyphosate tolerant crops is likely to cause emergence of resistant weeds, changes in soil microbial communities and losses in biodiversity.

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