Biosafety Briefing
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Recent biosafety information for events MON810, MON863, NK603 (maize) and GTS-40-3-2 (soya)

By **Third World Network**

This brief paper summarizes some of the latest findings regarding the safety of four transgenic events (MON810, MON863 and NK603 maize, and GTS-40-3-2 soya) that have been approved for human and animal consumption in many countries worldwide. Recent research has also shown new pieces of information related to their environmental impacts; however, only food/feed safety results and interpretations are discussed in this paper. The studies focus on the appearance of novel indications for human and animal toxicity, highlighting the need for updated consideration of this important information by the food safety and biosafety authorities. It also underlines the need for new scientific information to continuously inform the decision-making process, including consideration of review of decisions/approvals, as provided for under Article 12 of the Cartagena Protocol on Biosafety.

1. NK603 maize (tolerant to Roundup herbicide)

Novel proteins and other molecules

During the molecular characterization of NK603 it was shown that more novel transcripts could be created due to "read through", a process where RNA transcription proceeds through the terminator NOS 3'. This means that novel unintended RNA can be created from the transgene, adding uncertainty to gene regulation and potentially creating new proteins (that have not yet been characterized).

Read-through of terminator sequences is one source for the creation of dsRNA. dsRNA derives from the processing of larger RNA molecules through a number of pathways (Chong and Whitelaw, 2004; Lippman and Martienssen, 2004; Meister

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and Tuschl, 2004). It is usually observed by its ability to silence genes, that is, to stop the production of a protein by either preventing the production of mRNA or preventing the translation of mRNA. The potential to inadvertently create novel RNA regulatory molecules, usually in the form of dsRNA, is too high to ignore.

The following quote from 2006 Nobel Prize winner Craig C. Mello highlights the potential for dsRNA to produce silencing (RNA interference-RNAi):

"*[S]ilencing in response to a DNA transgene could still involve a dsRNA trigger: the transgene might integrate itself into the genome in such a way that a nearby promoter, or an inverted copy of the transgene itself, leads to the production of dsRNA, which could in turn enter directly into the RNAi pathway.*" (Mello and Conte Jr., 2004)

Herbicide toxicity

NK603 is a variety that has been developed to tolerate the application of the glyphosate-based herbicide formulation Roundup. Novel knowledge related to the health impacts of these herbicides has been brought to light by several authors in recent years. Roundup has been found to be highly toxic to human cells, at levels far below agricultural dilutions. This was found to affect hepatic, embryonic and placental cell lines, and human placental extracts, primary umbilical cord cells and freshly isolated testicular cells (Benachour and Séralini, 2009; Benachour et al., 2007; Clair et al., 2012; Gasnier et al., 2010; Richard et al., 2005). This knowledge should be included in the risk exposure for consumers of ingested NK603, as traces of herbicides or herbicide residues will be more likely to be found in derived food products.

In 2009, Benachour and Séralini showed that the adjuvants added to the commercial herbicide formulation played an important role in increasing the synergic toxicity of glyphosate. In their study, glyphosate mixed with common adjuvants (such as POEA-polyethoxylated talowamine) caused the death of human umbilical, embryonic and placental cells at much lower concentrations than glyphosate alone.

They also showed that the major glyphosate metabolite, aminomethylphosphonic acid (AMPA), is more stable in soil, plants and in food or feed residues, and is not only more toxic than glyphosate but also increases the toxicity of glyphosate or POEA in combination.

This study confirms results from other recent studies in animals and cell cultures pointing directly to health effects in humans as well as rodents and fish (following text from Traavik and Heinemann, 2007):

*"Female rats fed glyphosate during pregnancy demonstrated increased foetal mortality and malformations of the skeleton (Dallegrave et al., 2003). Nile Tilapia (*Oreochromis niloticus*) fed sublethal concentrations of Roundup exhibited a number of histopathological changes in various organs (Jiraungkoorskul et al., 2003). A study of Roundup effects on the first cell divisions of sea urchins (Marc et al., 2002) is of particular interest to human health. The experiments demonstrated cell division dysfunctions at the level of CDK1/Cyclin B activation. Considering the universality among species of the CDK1/Cyclin B cell regulator, these results question the safety of glyphosate and Roundup on human health."*

Another study (Axelrad et al., 2003) demonstrated the negative effect of glyphosate, as well as a number of other organophosphate pesticides, on nerve-cell differentiation. Surprisingly, in human placental cells, Roundup is always more toxic than its active ingredient, glyphosate. The effects of glyphosate and Roundup were tested at lower non-toxic concentrations on aromatase, the enzyme responsible for estrogen synthesis (Richard et al., 2005). The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation. The authors conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. They suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

In 2012, during in vitro experiments, it was described that Roundup herbicide provoked a decrease in testosterone levels in rats at very low concentrations (Clair et al., 2012). The same study also showed toxicity in testicular cells at higher concentrations.

Glyphosate has also been found to cause endocrine disruption in placental human cells at sub-agricultural doses within 24 hours, and in human cell lines (Richard et al., 2005; Gasnier et al., 2009).

Safety of whole food

In a recent study in 2009 by French researchers, the different results of genetically modified (GM) maize feeding trials were re-evaluated using a different analytical-statistical system than previously used (de Vendômois et al., 2009). The evaluation of the results for the NK603 feeding trial on rats revealed new side-effects linked to the consumption of the maize, which were sex- and dose-dependent.

Researchers found more significantly different physiological effects with male rats fed GM maize than with females, showing that repartition was sex-dependent. Differences were especially marked when male rats were fed at the higher percentage of GM maize (33%), showing the dose dependency. Differences were situated in liver and kidney parameters and urine phosphorus, relative lymphocyte and neutrophil differences.

The authors explain that the significant disturbances in the function of both liver and kidney cannot be dismissed as biologically insignificant. They conclude that their data suggest that this GM maize variety induces a state of hepatorenal toxicity and therefore ask for additional long-term (up to 2 years) animal feeding studies, preferably also multi-generational.

The latest research, which is long-term in nature (over the 2-year life span of rats), has shown that both NK603 maize and Roundup provoked chronic hormone and sex-dependent pathologies. Female mortality was 2-3 times higher mostly due to large mammary tumours and disabled pituitary function. Males suffered liver congestion and necrosis, severe kidney nephropathies and large palpable tumours. This may be due to an endocrine disruption mechanism linked to Roundup and a novel biochemical effect in the GM maize resulting from the transgene encoded EPSPS enzyme (Séralini et al., 2012).

2. GTS-40-3-2 soya (tolerant to Roundup herbicide)

Novel proteins and other molecules

A recent study of event 40-3-2 Roundup Ready soya was the first one to show the production of other transcripts, produced by a read-through of terminator NOS 3'. Just like for NK603, this means that novel unintended RNA can be created from the transgene, adding uncertainty to gene regulation, gene silencing and potentially creating new proteins (that have not been characterized).

In 2002, the developer argued that the only relevance of novel RNAs arising from transcription of the partial event or read-through of the terminator of the full-length insert would be a novel protein and they could neither detect a novel protein using specific molecular tools nor detect the effect of a novel protein from their feeding studies (Monsanto, 2002). However, subsequently researchers said that "these RNA variants [arising from read-through of the terminator in Roundup Ready soya] might code for (as yet undescribed) CP4 EPSPS fusion proteins" (Rang et al., 2005). Even more significantly, these researchers also found that "the read-through transcript was processed in four different RNA-variants" (Rang et al., 2005).

The authors of this study raised a significant concern that the NOS terminator sequence itself harbours a splice site, and therefore will have the same consequences in other GM plants containing this genetic element (a theory that is supported by recent results with NK603 and MON810).

"The cis regulatory regions that initiate and mediate splicing are located within the removed region of spliced transcripts. If this is also true for the mechanisms mediating post-transcriptional processing of the described variants, it seems reasonable to assume that the transcribed nos terminator region might be responsible for processing the RNA. Since the nos terminator was and still is commonly used as regulatory region in the production of genetically modified crops, read-through products and RNA variants could also be expressed in these plants." (Rang et al., 2005)

Herbicide toxicity

40-3-2 is a variety that has been developed to tolerate the application of glyphosate-based herbicide formulation Roundup. As explained for NK603 (see above for further information on the research studies demonstrating toxicity to human cells), there is novel knowledge related to the health impacts of these herbicides, knowledge that should be included in the risk exposure for consumers of ingested GM soya as traces of herbicides or herbicide residues will be more likely to be found in derived food products.

Safety of whole food

Several studies raise questions regarding the safety of the GM Roundup Ready soya that need to be further investigated:

Mice fed GM soya showed disturbed liver, pancreas and testes functions, as well as abnormally formed cell nuclei and nucleoli in liver cells, indicating increased metabolism and potentially altered patterns of gene expression (Malatesta et al., 2002; Malatesta et al., 2003; Vecchio

et al., 2004). Mice fed GM soya over their lifetime (24 months) showed more acute signs of ageing in the liver and lower metabolism, while the structure of liver cell nuclei suggests marked lowering of gene function (Malatesta et al., 2008). Rabbits fed GM soya showed enzyme function disturbances in the kidney and heart (Tudisco et al., 2006).

3. MON810 maize (insect-resistant)

Novel proteins and other molecules

Further molecular characterization of MON810 has shown important results. As previously explained for NK603 maize and 40-3-2 soya, MON810 has also been shown to produce variant RNA due to read-through of the NOS 3' terminator. The insertion or subsequent propagation of the event has resulted in a genomic deletion of the nos sequence. Researchers studying transcription of the event in MON810 found mRNAs that will code for unique Cry fusion proteins composed of the cry gene-determined amino acids and amino acids added at the end which are coded by the plant genome (Rosati et al., 2008).

Novel unintended RNA adds uncertainty to gene regulation, gene silencing and potentially creates new proteins (that have not been characterized). See above (NK603 maize and 40-3-2 soya) for details on effects of novel RNA molecules.

In another study, a comparison of a commercial maize variety carrying the MON810 event and its isogenic relatives (Zolla et al., 2008) indicates both that unanticipated changes occur as a result of the engineering process and that these can be more carefully characterized using profiling techniques that are not common in the scientific dossiers provided to regulators (Heinemann, 2007).

"[I]t is also evident that the insertion of a single gene does not result in a unique newly expressed protein, but rather in many differently expressed genes with respect to the control. This could be due to the fact that, when the transgene enters the nucleus, many genetic loci are randomly affected by the insertion procedure." (Zolla et al., 2008)

Heterogeneity in expression

There is no absolute relationship between the level of expression (transcripts or proteins) and the potential for a protein to cause harm. However, the exposure assessment needs to take into account real conditions of exposure to the novel protein. Current uses of averages are challenged by recent studies demonstrating the heterogeneity of protein expression. One study found significant differences in transgene expression levels between MON810 maize when analyzed at different growth stages, in different plant tissues and at two different locations within Germany (Nguyen and Jehle, 2007).

Safety of whole food

A recent study has found toxic effects in human liver cells during in vitro experiments exposing the protein produced by MON810, cry1Ab (Mesnage et al., 2012).

In November 2008, Italian researchers concluded that "the consumption of Bt MON810 maize…induced alterations in intestinal and peripheral immune response of weaning and old mice." "[T]hese results suggest the importance of considering the gut and peripheral

immune response to the whole GM crop, as well as the age, in the GMO [GM organism] safety evaluation" (Finamore et al., 2008).

In a more recent study, the different results of MON810 feeding trials were re-evaluated using a different analytical-statistical system than previously employed (de Vendômois et al., 2009). Results showed that GM-maize-linked effects are generally detected either after 14 weeks of consumption or at a high GM feed dose in the diet. Parameters affected relate to: blood cells, adrenal gland and kidney weights, an increase in blood urea nitrogen and higher spleen weight. In males, significant disturbed parameters were found in liver function. The authors ask for additional long-term (up to 2 years) animal feeding studies, preferably also multi-generational.

4. MON863 maize (insect-resistant)

Digestibility and allergenicity

MON863 produces a novel protein that is toxic to certain insects; this protein has been described by the developer as the cry3Bb1.11098 that is similar but not identical to cry3Bb1 (seven different changes have been shown). However, most of the studies performed by the company use cry3Bb1 and not cry3Bb1.11098. This situation could be misleading in terms of safety, particularly when taking into account results from the developer's study (MSL 17530) on the digestibility of cry3Bb1.11098.

MSL 17530 is a study on the digestibility or the resistance to digestion of the novel protein when eaten. These experiments are usually performed to study the allergenic potential of a protein. Even though this linkage has been criticized (Spok et al., 2005), these studies are still important to complete a hazard characterization.

Results showed that when using cry3Bb1.11098 in a simulated gastric fluid, a protein fragment of 57kDa remained for at least 24 hours. This is in contrast with cry3Bb1, where the protein fragment remained only for 2 minutes (when cry3Bb1 was produced in bacteria) or 15 minutes (when cry3Bb1 was produced in the maize). This demonstrates a real difference between the proteins, highlighting the need for different tests (such as acute toxicity tests) to be performed with the in-planta produced cry3Bb1.11098.

Safety of whole food

In May 2007, French researchers published their reanalysis of Monsanto data and concluded that there were indications of liver/kidney toxicity in rats fed Bt maize MON863 (Séralini et al., 2007). These results were also described in a more recent article (de Vendômois et al., 2009). The authors concluded that "In summary, the tendency for physiological disturbance is characteristic of almost all rats of all GM-fed treatment groups, and physio-pathological profiles differ according to dose or sex".

Note

The Biosafety Assessment Tool (www.bat.genok.org) was used as a support to gather information for this paper.

References

Axelrad, J.C., Howard, C.V. and McLean, W.G. (2003). The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon. *Toxicology* **185**, 67-78.

Benachour, N. and Séralini, G.E. (2009). Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chem Res Toxicol*. **22**(1), 97-105.

Benachour, N., Sipahutar, H., Moslemi, S., Gasnier, C., Travert, C. and Séralini, G-E. (2007). Time- and dose-dependent effects of Roundup on human embryonic and placental cells. *Arch Environ Contam Toxicol* **53**, 126-133.

Chong, S. and Whitelaw, E. (2004). Epigenetic germline inheritance. *Curr. Opin. Genet. Dev.* **14**, 692-696.

Clair E., Mesnage, R., Travert, C. and Séralini, G.É. (2012). A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels. *Toxicol In Vitro*. **26**(2), 269-279.

Dallegrave, E., Mantese, F.D.G., Coelho, R.S., Pereira, J.D., Dalsenter, P.R. and Langeloh, A. (2003). The teratogenic potential of the herbicide glyphosate-Roundup® in Wistar rats. *Toxicol. Lett.* **142**, 45-52.

de Vendômois, J., Rouiller, F., Cellier, D. and Séralini, G.E. (2009). A Comparison of the Effects of Three GM Corn Varieties on Mammalian Health. *Int. J. Biol. Sci.* **5**(7), 706-726.

Finamore, A., Roselli, M., Britti, S., Monastra, G., Ambra, R., Turrini, A. and Mengheri, E. (2008). Intestinal and Peripheral Immune Response to MON810 Maize Ingestion in Weaning and Old Mice. *J. Agric. Food Chem.* **56**, 11533-11539.

Gasnier, C., Benachour, N., Clair, E., Travert, C., Langlois, F., Laurant, C., Decroix-Laporte, C. and Séralini, G-E. (2010). Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. *J Occup Med Toxicol* **5**, 29.

Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.C. and Séralini, G-E. (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology* **262**, 184-191.

Heinemann, J.A. (2007). Letter to the Editor. *Environ Plann Law J* **24**, 157-160.

Jiraungkoorskul, W., Upatham, E.S., Kruatrachue, M., Sahaphong, S., Vichasri-Grams, S. and Pokethitiyook, P. (2003). Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). *Environ. Toxicol.* **18**, 260-267.

Lippman, Z. and Martienssen, R. (2004). The role of RNA interference in heterochromatic silencing. *Nature* **432**, 364-370.

Malatesta, M., Biggiogera, M., Manuali, E. et al. (2003). Fine structural analyses of pancreatic acinar cell nuclei from mice fed on genetically modified soybean. *Eur J Histochem* **47**, 385-388.

Malatesta, M., Boraldi, F., Annovi, G., Baldelli, B., Battistelli, S., Biggiogera, M. and Quaglino, D. (2008). A long-term study on female mice fed on a genetically modified soybean: effects on liver ageing. *Histochem Cell Biol* 2008 Jul 22, 18648843.

Malatesta, M., Caporaloni, C., Gavaudon, S. et al. (2002). Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. *Cell Struct Function* **27**, 173-180.

Marc, J., Mulner-Lorillon, O., Boulben, S., Hureau, D., Durand, G. and Belle, R. (2002). Pesticide Roundup Provokes Cell Division Dysfunction at the Level of CDK1/Cyclin B Activation. *Chem. Res. Toxicol.* **15**, 326-331.

Meister, G. and Tuschl, T. (2004). Mechanisms of gene silencing by double-stranded RNA. *Nature* **431**, 343-349.

Mello, C.C. and Conte Jr., D. (2004). Revealing the world of RNA interference. *Nature* **432**, 338-342.

Mesnage, R., Clair, E., Gress, S., Then, C., Székács, A. and Séralini, G-E. (2012). Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. DOI 10.1002/jat.2712.

Monsanto (2002). Additional Characterization and Safety Assessment of the DNA Sequence Flanking the 3′ End of the Functional Insert of Roundup Ready® Soybean Event 40-3-2. MSL-17632. Monsanto Company. http://www.food.gov.uk/multimedia/pdfs/RRSsafetysummary.pdf.

Nguyen, H.T. and Jehle, J.A. (2007). Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. *J Plant Dis Protect* **114**, 82-87.

Rang, A., Linke, B. and Jansen, B. (2005). Detection of RNA variants transcribed from the transgene in Roundup Ready soybean. *Eur. Food Res. Technol.* **220**, 438-443.

Richard, S., Moslemi, S., Sipahutar, H., Benachour, N. and Seralini, G.E. (2005). Differential Effects of Glyphosate and Roundup on Human Placental Cells and Aromatase. *Environ. Health Perspect.* **113**, 716.

Rosati, A., Bogani, P., Santarlasci, A. and Buiatti, M. (2008). Characterisation of 3' transgene insertion site and derived mRNAs in MON810 YieldGard maize. *Plant Mol. Biol.* **67**, 271-281.

Séralini, G-E., Cellier, D. and de Vendomois, J. (2007). New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. *Arch. Environ. Contam. Toxicol.* **DOI: 10.1007/s00244-006-0149-5**, 596-602.

Séralini, G-E., Clair, E., Mesnage, R., Gress, S., Defarge, N., Malatesta, M., Hennequin, D. and de Vendômois, J. (2012). Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Food Chem. Toxicol.* http://dx.doi.org/10.1016/j.fct.2012.08.005.

Spok, A., Gaugitsch, H., Laffer, S., Pauli, G., Saito, H., Sampson, H., Sibanda, E., Thomas, W., van Hage, M. and Valenta, R. (2005). Suggestions for the assessment of the allergenic potential of genetically modified organisms. *Int. Arch. Allergy Immunol.* **137**, 167-180.

Traavik, T. and Heinemann, J.A. (2007). *Genetic Engineering and Omitted Health Research: Still No Answers to Ageing Questions*. Third World Network Biotechnology & Biosafety Series No. 7. ISBN: 978-983-2729-76-1.

Tudisco, R., Lombardi, P., Bovera, F., d'Angelo, D., Cutrignelli, M.I., Mastellone, V., Terzi, V., Avallone, L. and Infascelli, F. (2006). Genetically modified soya bean in rabbit feeding: detection of DNA fragments and evaluation of metabolic effects by enzymatic analysis. *Anim Sci* **82**, 193-199.

Vecchio, L., Cisterna, B., Malatesta, M. et al. (2004). Ultrastructural analysis of testes from mice fed on genetically modified soybean. *Eur J Histochem* **48**, 449-454.

Zolla, L., Rinalducci, S., Antonioli, P. and Righetti, P.G. (2008). Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. *J Proteome Res* **7**, 1850-1861.