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Testbiotech e. V.
Institute for Independent
Impact Assessment in
Biotechnology

High levels of residues from spraying with glyphosate found in soybeans in Argentina



Photo: Testbiotech, Northern Argentina, April 2013

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Summary

In April 2013, Testbiotech took samples of soybeans from fields in Argentina in regions that are known for the cultivation of genetically engineered soybeans. The samples were taken shortly before the harvest was due. Nearly all the soybeans grown in Argentina are genetically engineered, and made resistant to the herbicide glyphosate (brands such as Roundup). These soybeans were originally developed by the US company, Monsanto. Currently there are only very few publications on the actual load of residues in these genetically engineered plants after they have been harvested. So the purpose of this pilot project was to gather some more data on residues from spraying with glyphosate.

The samples were analysed in a laboratory at the University of Buenos Aires. The results showed a surprisingly high content of residue of up to almost 100 mg /kg. In seven of the eleven samples the level was higher than the international maximum residue level (MRL) of 20 mg/ kg allowed in soybeans products used for food and feed. The results were confirmed in a second analysis. Aware that these results were alarmingly high, Testbiotech decided to publish its findings despite the small number of samples.

Testbiotech believes the high level of residues from spraying found in the soybeans indicates that they were not grown under conditions conforming to environmentally friendly agricultural practice. The dosage of glyphosate used in the fields concerned is likely to be much higher than recommended. Such high dosages could have been due to increasing weed resistance to the herbicide glyphosate which is also reported in Argentina.

Over-usage of glyphosate mixtures can have a negative impact on the environment and rural communities. A high level of residues from spraying can also impact health at the food and feed consumption level.

Similar problems with the application of high dosages of glyphosate are also likely to occur in countries such as Brazil and the US where these genetically engineered soybeans and other glyphosate resistant crop plants are grown on large scale, and an increasing number of herbicide resistant weed species are being reported.

Testbiotech recommends close monitoring of herbicide applications in those regions where the herbicide resistant plants are grown. This monitoring should cover residues in soil and water as well as in blood and urine samples from farmers, rural communities and livestock. Further, any soybean products containing residues from spraying which are used as food and feed should be subjected to many more controls.

The health risks and the environmental impact of glyphosate and its mixtures needs to be reassessed. There should be a substantial reduction in the high maximum residue levels currently allowed in food and feed products.

Agricultural practice should also be changed, switching from growing herbicide resistant plants to agriculture practice that supports crop diversity and biodiversity in the fields as well as in the rural areas.

1. Why Testbiotech started this project

The reason Testbiotech started this pilot project were publications reporting a higher usage of glyphosate due to herbicide resistant weeds in genetically engineered soybean fields (Benbrook, 2012). There is, in addition, a severe lack of publications on the load of residues in the plants (Kleter et al., 2011). Furthermore there have been several reports in the media about the impact on health of glyphosate usage in Argentina¹. Our aim was gather more data on the actual residues in the soybeans. We have published our preliminary findings due to alarmingly high concentrations found in the samples.

2. When and where we collected the material



From 16 to 19 April 2013 Testbiotech collected eleven samples of genetically engineered soybeans in Argentina.

The fields where we collected the beans are in the districts of Las Lajitas, Joaquín V. González, and Metán in the province of Salta, in the North of Argentina (see map). Nearly all the soybeans cultivated in Argentina, are genetically engineered to be resistant to the herbicide glyphosate². This also applies to the region where the samples were taken.

The samples were composite samples and consisted of around 200 mg soybeans from around a dozen plants from each field.

The soybeans were almost ready to harvest, but some of them still had a greenish colour.

3. How the soybeans were analysed

The beans were sent to a laboratory at the University of Buenos Aires, where they were dried (48 h at 60°C) and analysed with HPLC chromatography³. Since the results showed unexpectedly high levels of glyphosate and its metabolite AMPA, we asked the laboratory to conduct a second analysis with some samples, and also to determine the degree of moisture in the beans. Analyses were performed in June and September 2013.

¹ See for example www.nabu.de/themen/gentechnik/anbauundfreisetzung/sonstigenutzpflanzen/13327.html

² www.isaaa.org

³ Analytical method (from the laboratory protocol): Glyphosate (PMG) and aminomethylphosphonic acid (AMPA) determination in soybean samples were made by HPLC using a UV-Vis detector, 250 µl injection loop, mobile phase of ammonia acetate (3.5 mM)/acetonitrile, a gradient method and a flow of 0,7mL/min. The analytical method requires derivatization by FMOC.

4. What were the results?

We found that seven of the eleven samples had a much higher maximum residue level than 20 mg. The highest residue level found was almost 100 mg/kg. The results from June 2013 were confirmed by a further analysis of five of the same samples conducted in September 2013 (with the exemption of the sample M3 which might be an outlier). The results also show that between June and September the sum of residue had not changed significantly during storage.

Table: Overview of results (numbers in bold exceed MRL of 20 mg/kg)

#	Residue (mg/kg), June 2013			Residues (mg/kg), Sept. 2013		
	Glyphosate (acid)	AMPA	Glyphosate (Sum)	Glyphosate (acid)	AMPA	Glyphosate (Sum)
M1	5,3	<0,05	<5,34			
M2	7,4	6	16,54	1,4	10	16,63
M3	11,6	<0,05	<11,67	7,5	46	77,54
M4	22,5	18,1	50,06			
M5	18,8	13,7	39,66			
M6	11	13,2	31,10	12	12	30,27
M7	19,4	22,6	53,81			
M8	11,3	23,6	47,23			
M9	25,8	47	97,36	16,2	52,5	96,14
M10	14,3	<0,05	<14,38			
M11	23,9	33,8	75,36	4	46,5	74,80

AMPA has a molecular weight of 111,04, Glyphosate has a molecular weight of 169,07, the AMPA residues were thus calculated with a factor of 1,52 for generate a Glyphosate equivalent. (Glyphosate acid + (AMPA*1,52) = Glyphosate (sum)). Moisture of the samples was around 6 percent.

5. How to interpret the data?

The currently high maximum residue level of 20mg/kg for soybeans was proposed by a working group organised by the Food and Agricultural Organisation of the UN (FAO). This level was set on the basis of data from field trials: Soybeans were cultivated using herbicide applications with glyphosate as recommended by the producers. These trials showed that under normal agricultural conditions, the level of 20 mg/kg in the harvest will not be exceeded. In consequence this was defined as the maximum residue level (see FAO, 2005) which is also valid for the EU⁴. In general, 20 mg /kg is a very high maximum residue level for food and feed products. For other herbicides there is normally a residue level of 0,1 mg /kg in the EU. This extremely high maximum residue level for glyphosate was allowed because it is considered as showing

⁴ http://ec.europa.eu/sanco_pesticides/public/?event=homepage&CFID=21231774&CFTOKEN=bb3c828a98e540bf-8E1CDCE0-E247-6717-70004ADD68CF82CD&jsessionid=09045d3f55190380240f5d1b28596c4b67e4TR

low toxicity – an assumption that is now under discussion and disputed controversially (see below).

To draw conclusions from our data, the most relevant metabolite AMPA (Aminomethylphosphonic Acid) which is most relevant metabolite from degradation of glyphosate in the plants also has to be taken into account. AMPA is assumed to have a similar toxicity to glyphosate (see FAO, 2005). Therefore the sum of detected glyphosate has to be calculated considering the molecular weight of the two substances (see table of results). The results showed that seven of the eleven samples had a residue load higher than the Maximum Residue Level (MRL) and one of those samples had a content of about 100 mg/kg which is five times higher than the MRL.

In coming to conclusions from our own findings, it has to be acknowledged that the formulas of glyphosate applied in the fields are always a mixture. Especially additives such as Polyethoxylated tallow amine (POEA) are a matter of concern: These tallow amines are supposed to be much more toxic than glyphosate alone (see below). POEA are added to many mixtures of glyphosate to enhance the efficacy of the herbicide. It can be added in concentrations of up to 20 percent and more (see Then, 2011). Consequently, the mixtures applied in the fields have a much higher toxicity than glyphosate alone. It has to be assumed that a high level of residues from glyphosate is also an indication of high dosages of toxic additives such as tallow amines being applied in the fields.

6. What conclusion can be drawn ?

We are not aware of any publication reporting similar findings. Since we know that this kind of data is rare (see Kleter et al., 2011), the first conclusion from our project is that there should be a lot more investigation carried out in areas where these genetically engineered plants are cultivated.

Further, Testbiotech is of the opinion that the high level of residues found in the plants by far exceeds the maximum residue level for soybeans used in food and feed and are an alarm signal:

- The high level of residues found in the soybeans strongly indicates that these plants were grown under extreme agricultural conditions. It is very likely that the amounts of glyphosate-based herbicides applied to the plants were much higher than recommended. It is likely that emergence of herbicide resistant weeds such as the johnsongras (*Sorghum halepense*) are a main reason for this. These resistant weeds are especially a problem in the region of Salta (Binimelis et al., 2009).
- Because of the very high dosage of herbicides being applied, the environmental impact of cultivating these soybeans is likely to be much higher than assumed so far.
- Also the health risks for the users and the rural communities can be supposed to be higher than assumed under “normal” conditions. In the light of these findings, media reports on the negative impact on the

health of people living in those regions where the soybeans are grown become highly relevant.

- Finally the impact on health from the soybeans being fed to animals or consumed by humans can be significant either alone or in combination with other factors. It has to be expected that these soybeans also have high levels of residues from additives such as tallow amines.

We cannot rule out that the soybeans from the fields we took samples from will be mixed with other soybeans to dilute the load of residues before using them as food or feed. This would mask the very high concentration of residues. Such mixtures of soybean products containing material with residue levels that are too high could also be imported into the EU or other regions. The only way to prevent this is to impose systematic controls in the fields and throughout the production chain.

7. Background:

7.1 What is glyphosate and how is it connected to genetic engineering?

Glyphosate is broad spectrum herbicide (known under brands such as Roundup) which is toxic to weeds and agricultural plants. Around 20 years ago, Monsanto managed to insert a bacterial DNA into crop plants making the plants to resistant spraying with glyphosate. This technology is used in soybeans, maize (corn), oilseed rape, sugar beet, alfalfa and cotton. Genetically engineered crops make it possible to apply glyphosate widely on the fields.

This practice is considered to be the main reason for increasing number of herbicide resistant weeds. For example over recent years, the 'Weedscience' database (<http://www.weedscience.org>) has increasingly recorded the emergence of new resistant weeds across the United States. Either these weeds can no longer be eradicated using glyphosate, or greater quantities have to be applied to do so. In the United States, as of October 2012, 13 resistant weeds had been recorded in 31 states. There are also reports about herbicide resistant weeds such as the johnsongras (*Sorghum halepense*) in Argentina (Binimelis et al., 2009).

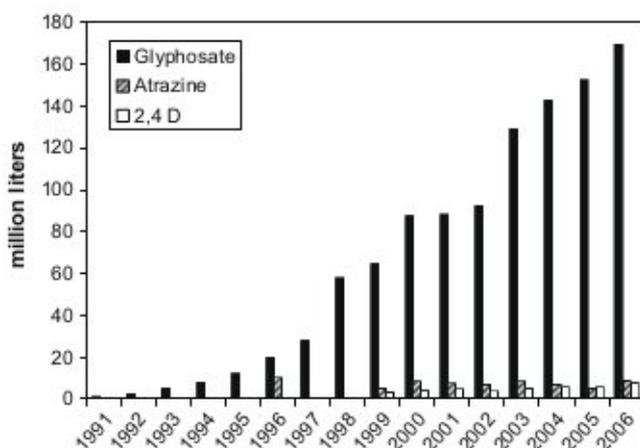


Figure 1: Applications of glyphosate are strongly increasing in Argentina (Binimelis et al., 2009)

Benbrook (2012) estimates that herbicide-resistant weeds have already blighted around 20 to 25 million hectares of arable land in the United States. According to Benbrook (2012), in the period from 1996 to 2011, herbicide application on herbicide-resistant crops increased by 239 million kg and 70% of this increase is attributable to the cultivation of genetically engineered soybeans. Binimelis et al (2009) report about strongly increasing glyphosate applications in Argentina between 1996 and 2006.

7.2 What is the environmental impact of glyphosate applications?

The European Food Safety Authority (EFSA) has confirmed that the cultivation of genetically engineered herbicide resistant plants is problematic for the environment. In one of its opinions published in 2012, on the cultivation of Roundup Ready soybeans (technical name: 40-3-2) which are also grown in Argentina, it says:

“The EFSA GMO Panel is of the opinion that potential adverse environmental effects of the cultivation of soybean 40-3-2 are associated with the use of the complementary glyphosate-based herbicide regimes. These potential adverse environmental effects could, under certain conditions, comprise: (1) a reduction in farmland biodiversity; (2) changes in weed community diversity due to weed shifts; (3) the selection of glyphosate resistant weeds; and (4) changes in soil microbial communities.” (EFSA, 2012)

The cultivation of herbicide resistant soybeans is indeed responsible for declining biodiversity on farmland. For example, the Monarch butterfly, an icon of nature conservation in the USA, migrates between the USA and Mexico, where the butterflies hibernate. It has been observed that the size of the butterfly population arriving in Mexico over the last 10 years has fallen significantly. One reason for this is that the number of forage crops, which are important for the caterpillars (certain milkweed species), has declined considerably in the US mostly due to large scale cultivation of the genetically engineered soybeans being grown (Pleasants & Oberhauser, 2012).

It is also known that glyphosate application has particular effects on aquatic ecosystems (FAO, 2000). Even in small concentrations it can have adverse effects on aquatic life. Studies of amphibians highlighted significant toxicity. Frog and toad tadpoles (Relyea, 2005 a and b; Relyea 2012; Relyea & Jones, 2009) are just as sensitive to the presence of glyphosate in water as frog embryos (Paganelli et al., 2010, see also Wagner et al., 2013). According to information from the US EPA, glyphosate is a threat to the habitat of protected amphibians such as the red-legged frog⁵.

⁵ http://www.epa.gov/oppsrrd1/registration_review/glyphosate/index.htm
<http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2009-0361-0003>

Roundup and, in particular, the additive POE tallow amine have also proved to be toxic to freshwater mollusks (Bringolf et al 2007). The level of toxicity of POE tallow amine in fish has been recorded as being 30 times higher than the level of glyphosate present in the same fish (Servizi et al., 1987, quoted by PAN AP, 2009).

The negative effects of the cultivation of glyphosate tolerant crops actually affect rural areas as a whole, rather than only agriculture. 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.

7.3 What about risks to human health?

Usage of products derived from herbicide tolerant crop means that consumers are exposed to a specific pattern of residues from spraying: The residues and metabolites of these herbicides become permanent constituents in the resulting food and feed.

In addition, pesticides like Roundup often contain additives such as polyethoxylated tallow amine (POEA), thought to foster better absorption of the toxins by the crops thereby enhancing their effectiveness. These tallow amines are far more toxic than glyphosate. Their use in German agriculture has therefore been significantly restricted⁶, unlike other countries.

Despite the large-scale use of pesticides in growing genetically engineered plants, there is an astounding lack of data relating to residue controls. According to Kleter et al. (2011), there is almost a complete lack of data on residue levels in GE crops:

"While residue data from experimental studies have been used to establish the residue tolerances for the herbicide-crop combinations described above, it would be interesting to compare these tolerances with what is actually measured in the field, i.e. in commercially produced foods. No measurement of the herbicides of interest in the particular crop foods in question is apparently carried out by the centralised or federal pesticide residue monitoring programmes of the EU, the United States and Canada."

The European Food Safety Agency, EFSA, (2011) is assuming that certain residues can be found on a regular basis in the human bloodstream (although there are many other ways of coming into contact with the toxins than by consuming GE crops). In a review of a Canadian publication that stated that residues and metabolites of glyphosate, such as MPPA, had also been found in the bloodstream of pregnant women (Aris & LeBlanc, 2011), EFSA states that

⁶ http://www.bvl.bund.de/DE/04_Pflanzenschutzmittel/05_Fachmeldungen/2010/2010_06_01_Fa_anwendungsbestimmungen_tallowamin-Mittel.html

these findings are not unexpected:

“From the consumer health perspective, the observations described by the authors on the presence of glyphosate and glufosinate in non-pregnant women blood (5% and 18% of the subjects, respectively) and of 3-MPPA in non-pregnant women, pregnant women and the fetal cord blood are not unexpected. It is known that pesticides are generally well absorbed by the gastrointestinal tract and that an exposure to the two herbicides investigated through the consumption of food commodities is plausible.”

An investigation by Friends of the Earth (2013)⁷ found glyphosate to be present in many samples of urine from EU citizens. To which extent genetically engineered plants are the reason for this observation, is not clear, since there are many opportunities to come into contact with the herbicide. It is also used increasingly in conventional agriculture and even in private house gardens.

Exposure to residues from spraying with these herbicides can, even in small concentrations, affect hormone metabolism (see for example Gasnier et al., 2009; Thongprakaisang et al., 2013), and disrupt, for example, embryo development or influence cell division and cancer growth. There have been a series of papers published on glyphosate and glyphosate mixtures, which show such effects to be plausible. In many cases combinatorial effects were found between glyphosate and additives such as tallow amine. As mentioned, the toxicity of the herbicide formulas used in practice, show a higher toxicity than glyphosate alone (Mesnage et al., 2012; Kim et al., 2013). It should also be taken into account that there might be synergies between naturally occurring estrogens in soybeans and the residues from spraying with potential hormonal activity.⁸

It is a matter of concern that, according to the German Institute for Risk Assessment (BfR 2012), only one long-term study focusing on a Roundup product that is readily available on the market has been conducted (Seralini et al., 2012). This study (Seralini et al., 2012), led to controversial discussions because it pointed to a significantly higher health risk for rats that were exposed to low levels of Roundup throughout their lifetime. In 2013 another study was published (Krüger et al.) which showed that there were some signs of adverse health effects in Danish dairy cows all of which had glyphosate with their urine.

Constant exposure to herbicide residues, such as glyphosate, can also have an indirect impact on health, for example, it might cause changes in the intestinal flora of humans, thereby increasing the risk of developing illnesses.

Glyphosate is effective against certain bacteria, such as *E. coli* (Forlani et al., 1997; Carlisle & Trevors, 1986), and can, in high concentrations, damage the intestinal flora of cattle (Reuter et al., 2007). Even low doses impact the microbial flora of poultry and there is a reduction in the number of beneficial microbes (Shebata et al., 2012).

⁷ www.bund.net/fileadmin/bundnet/pdfs/gentechnik/130612_gentechnik_bund_glyphosat_urin_analyse.pdf

⁸ This is also discussed in a legal challenge by Testbiotech on the market authorisation of genetically engineered soybeans, www.testbiotech.org/en/eugericht

8. Recommendations

- The maximum residue levels for glyphosate should be lowered substantially.
- There should be close monitoring of glyphosate applications and concentrations in those regions where the herbicide resistant plants are grown. This monitoring should cover residues in soil and water as well as in blood and urine samples taken from farmers, rural communities and livestock.
- Importing countries should rigorously monitor residue loads from spraying.
- Additives such as tallow amines should be forbidden, the exact formulations of herbicides should be made public.
- Cultivation of herbicide resistant plants should not be allowed in Europe.
- In those countries where these herbicide resistant plants are grown, agricultural practice should be changed, switching to agricultural practice that supports crop diversity and biodiversity in the fields as well as in the rural areas.
- The health risks and the environmental impact of glyphosate and its mixtures should be reassessed.

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