Feeding study with genetically engineered maize NK603 does not provide evidence of adverse effects on the health of rats

But the debate continues on how to assess health risks associated with GMOs

17 April 2018 / An EU-funded research project known as G-TwYST conducted a two-year feeding trial with rats using genetically engineered maize resistant to glyphosate (NK603). According to the results which are not yet finally published, the diet fed to the rats did not trigger any clear signs of health effects. The study followed internationally agreed standards. However, it is not fully comparable with a previous rat feeding study using the same maize line: the G-TwYST study used a different rat strain and was designed differently to the original study. In the previous study, the outcome was interpreted as triggering severe health effects in the rats.

In general, this study does not allow any conclusions to be drawn on the safety of food derived from genetically engineered plants; and open questions remain in the specific case of NK03. One of them concerns the specific batch of genetically engineered maize used in the G-TwYST study. Under practical growing conditions, maize is sprayed more frequently and with higher dosages than was the case in this study. This can lead to higher amounts of residues in the harvest and also changes in plant constituents. But the maize as used in the trials showed very low levels of residues from spraying with glyphosate. There is also one very surprising result: The animals in one of the groups fed with the genetically engineered maize showed a significantly higher body weight.

In the EU, around 60 different lines of genetically engineered plants are already allowed for import and use in food and feed; many of them were never tested in short- or long-term feeding studies to investigate health risks. Most of these plants are resistant to not just one herbicide, but have been engineered to be resistant to several herbicides and, in addition, express insecticidal toxins. Many experts have also raised doubts about whether the feeding studies as performed are sensitive enough to examine the risks. Currently, there is no generally accepted method to assess the factual health risks for humans.

Consequently, this feeding trial is of rather limited value when it comes to the overall risks of introducing genetically engineered plants into the food chain.

The framework and experts involved

The G-TwYST project started in 2014 and ends in 2018. It is funded by the EU Commission and conducted in connection with other EU research projects, such as GRACE, MARLON, PRESTO and PRICE. Within these projects, research is dominated by a specific group of scientists, who all have a well-established relationship with each other. These experts were involved in many of the projects, either as projects leaders or leading experts. As analysis carried out by Testbiotech shows, many experts also had affiliations with industry or organisations funded by industry (Testbiotech, 2015). For example, the coordinator of G-TwYST, Pablo Steinberg, who also participated in the EU research projects GRACE and MARLON, had affiliations with institutions, such as the International Life Sciences Institute (ILSI) that is funded by food and agrochemical companies. ILSI is known for "co-opting academic contacts, infiltrating major scientific bodies and medical associations, and influencing the generation of scientific evidence” (Sacks et al., 2018). This is relevant since any influence of companies with an interest in the marketing of genetically engineered plants should be strictly avoided in order to safeguard publicly funded risk research on relevant products.
Various stakeholders, including civil society organisations, consumer protection, animal welfare and environment protection groups, were invited to discuss the findings. However, these organisations were not invited to participate in relevant steps of the project, such as planning, the selection of the scientists or in conducting the study. Overall participation of these groups was at a low level or not established at all.

Starting point and aim of the project

The objective of the "GMP Two Year Safety Testing" research project (G-TwYST) was to test the health effects of transgenic maize NK603 in a 90-day feeding trial, and in a combined one- and two-year feeding trial (www.g-twyst.eu [1]). NK603 is produced by Monsanto and is resistant to applications of glyphosate. It is not authorised for cultivation in the EU.
The starting point for this project was a study conducted by the French scientist Gilles-Éric Séralini published in 2012. Séralini found an increase in the incidence of tumours when rats were fed with maize NK603 and / or exposed to glyphosate in a two-year feeding trial. Several authorities, including the European Food Safety Authority, declared that the study was flawed. Criticism was directed at the very low number of rats used in the trials and the genetic background of the rats that were used (Harlan Sprague–Dawley rats), which are known to develop spontaneous tumours. The study was withdrawn from the scientific journal, Food and Chemical Toxicology after active involvement of experts affiliated with Monsanto (Retractionwatch, 2017). In 2014, the Séralini study was re-published in the open-access journal Environmental Sciences Europe (Seralini et al., 2014). In 2017, another publication by the same group could not confirm the pathological findings at the level of transcriptome. (Mesnage et al., 2017).
The aim of G-TwYST was to conduct a comparable long-term feeding study with NK603 to assess its health effects.

Methodology and findings

Pellets derived from NK603 maize, cultivated with and without spraying of glyphosate, harvested in Canada in 2014, was fed to rats for a period of over two years, starting from April 2015. The strain of rats used in the trials (Wistar) have different genetic background to those used by the Séralini team (Harlan Sprague–Dawley rats) and are not so likely to spontaneously develop tumours. More rats were used in this new study and several statistical methods were used to assess the data. In addition, a histopathological report was prepared and a paper on the composition of the maize was published. Unlike the research conducted by Séralini et al., glyphosate was not administered separately to an additional group of rats.
No treatment-related findings were identified by the G-TwYST team. However, there is also one very surprising result: The animals in one of the groups fed with the genetically engineered maize consistently showed a significantly higher body weight .
Overall, the G-TwYST team applied standard methods that were mostly in compliance with the relevant OECD standards - this strengthens the robustness of the study. However, for several reasons mentioned above, the outcome of the study is only partially comparable to the one conducted by the Séralini group.

Strengths and weaknesses

The diets that were tested did not induce treatment-related health effects. The outcome of this study might not be conclusive for potential health risks associated with the consumption of NK603, but it should be taken into account in its overall risk assessment. Therefore, we welcome the fact that the Séralini et al. publication triggered a second in-depth feeding study with the same event. We would conclude that NK603 is now one of the genetically engineered plants that have been subjected to more detailed investigation than others.

However, there are also some weaknesses:

(1) It might have been helpful to include Harlan Sprague-Dawley rats in the trial. While this strain of rats is known to develop spontaneous tumours (which can become a severe limitation for evaluation of the trials), it nevertheless might have been suitable for detecting effects which are only subtle, but of relevance when it comes to overall risks for human health. In this context, we would not consider one of the two strains of rats superior to the other, but just different.

(2) In the study design, the maize cultivated in Canada and used for the purposes of the study is not necessarily representative of NK603 that has been grown under practical conditions. The maize was only sprayed once with glyphosate with a dosage of 1.35 kg / hectare about one month after sowing. As the feed analysis shows, the residues from spraying with glyphosate were on a low level, below 0,5 mg / kg. Under practical conditions, glyphosate-tolerant maize is very often sprayed with the complementary herbicide more than once and with higher dosages per hectare. The application of

the complementary herbicide can influence the load of residues and the composition of biologically active substances in the plants. Therefore, the dosage and the number of applications of the complementary herbicide is relevant for assessing potential adverse health effects triggered by consumption of the maize.

(3) Although the plants were sprayed with glyphosate (which strengthens the study), potential adverse health effects associated with the consumption of the sprayed maize might have been overlooked in this trial. The reasons for this are the relatively low dosage applied in the field and the following processes of degradation caused through production of the pellets, as well as the storage of the feed for an overall period of more than two years. The Séralini team avoided some of these problems by running an additional test group that was exposed to defined dosages of glyphosate. In the light of continuing discussions on the health risks of glyphosate, such additional data would have added a lot of value to the study.

(4) The outcome of this study cannot be seen as representative for the overall health risks of genetically engineered plants in the food chain. This is for several reasons: NK603 is not representative of current large-scale cultivation; NK603 was brought onto market for the first time around 20 years ago. Currently, this event does not play a major role in maize production worldwide. It is the so-called stacked events which inherit a combination of several gene constructs and traits (such as herbicide tolerance and production of insecticidal proteins) that are of relevance in this respect. Thus, the likelihood of humans or animals being exposed to NK603 as a single event is very low. The stacked events are much more relevant – and these can have a combination of up to six insecticidal proteins. At the same time, they can be engineered to inherit several genes making them resistant to herbicides, such as glyphosate, glufosinate, dicamba and 2,4-D. These plants are also authorised for import into the EU. However, none of them have ever been tested for long-term health effects. Most of them were not tested at all in feeding studies designed to investigate health effects. And no research has been conducted to assess the combinatorial effects of these plants mixed into one diet. There are already 31 lines of GE maize (events) authorised for import into the EU, in addition to 32 events from other plants species, such as soybeans, oilseed rape, cotton seeds and sugar beet. Nevertheless, the combinatorial effects of mixtures used in the diet are not subjected to risk assessment in the EU.

Furthermore, there are very few studies on the monitoring of health effects under practical conditions, such as the one conducted by a former Monsanto staff member, van Eenennaam (Van Eenennaam & Young, 2014). However, this study has substantial flaws. For example, it does not take into account the effects of breeding programs and is designed to enhance production, as well as changes in the housing of the animals and standards of hygiene. Thus, it can be expected that potential effects from feed will be masked by other much more influential impact factors, especially if no groups for comparison are incorporated.

Do we need feeding studies at all?

Many experts have also raised doubts about whether the feeding studies as performed are sensitive enough to examine the risks. Currently, there is no generally accepted method to assess the factual health risks for humans. Indeed, feeding trials with rats might not be the best method to detect potential adverse health effects of genetically engineered plants. The composition of these plants is not as clearly defined as specific chemical compounds, and the mechanisms that can cause negative health effects can be various, such as altered plant composition, effects of intended additional proteins or any unintended gene products. In general, most potential health effects due to genetically engineered plants are much more difficult to investigate compared to plants composed of defined chemical substances.

However, feeding trials are so far the only method frequently used to not only assess single isolated compounds, but also the whole food and feed derived from these plants. Further, feeding trials with whole feed are carried out with poultry, which normally last for a period of 42 days. However, these trials are only meant to provide information about the nutritional quality of the feed and cannot provide reliable information on health effects. Only experts with specific interests might try to use such data to assess potential health effects (see, for example, Van Eenennaam & Young, 2014).

90-day feeding trials are certainly not sufficient in regard to the complexity of the risks they are used to assess, but at least they can deliver some basic data that can inform further risk assessment. This is the reason why in 2013, the EU Commission (EU Commission, 2013) made such feeding trials mandatory for market applications filed after the beginning of 2014 (but stacked events derived from crossing of genetically engineered plants are excluded).

Apparently, several of the experts and coordinators involved in projects such as GRACE and G-TwYST had very clear expectations in this regard, and repeatedly stated at the meetings with stakeholders that it would not be necessary to have feeding trials studies as a mandatory element of risk assessment.

Currently, around 60 genetically engineered events have been assessed and authorised for import into the EU. Many of those were never tested in 90-day feeding trials. One example is the genetically engineered maize known as SmartStax, which produces six insecticides and is engineered to be resistant to two herbicides. The EU Commission issued market authorisation for this stacked event without requesting any feeding trials with whole food and feed to assess potential health effects. It should further be noted that the combinatorial effects of genetically engineered plants mixed into food and feed have likewise never been assessed.

There are further levels of complexity that will add to these problems in the near future: Market applications for so-called stacked events, such as SmartStax, are increasing. In addition, several applications have been filed for plants that are changed in their nutritional quality. The risk assessment of these plants might prove to be much more complicated than for plants that were only made resistant to one herbicide.

The complexity of the underlying scientific problems cannot be answered at the present time. Basically, there are two scenarios for decision-making on future developments:

- One possible solution is to reduce uncertainties by stopping, or at least substantially reducing the number of market authorisations. Testbiotech strongly recommends this approach.
- Developing better methods for assessing health impacts to generate more reliable results. This could mean making feeding trials over the lifetime of the animals and following generations compulsory. At the same time, much more effort must be put into developing more reliable methods that can be used to complement or replace feeding studies.

In any case, there must be more research organised to include real participation of civil society and which prioritises the protection of human health and the environment - all of which must be completely independent of industry. Research organised by the EU within the framework of GRACE and G-TwYST does not fulfil these preconditions, nor is this the case for previous studies such as those summarised under previous research projects from 2001-2010 outlined by the EU Commission (EU Commission, 2010).

Conclusions and outlook

Ultimately, the feeding trial is notable and relevant for the tested diet. But it is of rather limited value, when it comes to the overall risks triggered by the introduction of genetically engineered plants into the food chain. Moreover, there is currently no strategy in the EU on how to reliably assess the potential long-term effects of the consumption of these plants.

In general, contrary to what might be expected from the legal framework of the EU, the EU Commission is not able to sufficiently ensure the safety of products entering the food chain in Europe. While it is not likely that each of these products will trigger severe health effects in each case, the overall health risks remain a matter of huge uncertainty.

Currently, there is no research program in the EU which gives priority to the precautionary principle and the protection goals, such as health and the environment, when it comes to regulated products and technologies, such as genetically engineered organisms i.e. 'GMOs'.

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Links
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[2] https://publications.europa.eu/en/publication-detail/-/publication/d1be9ff9-f3fa-4f3c-86a5-beb0882e0e65/language-en
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