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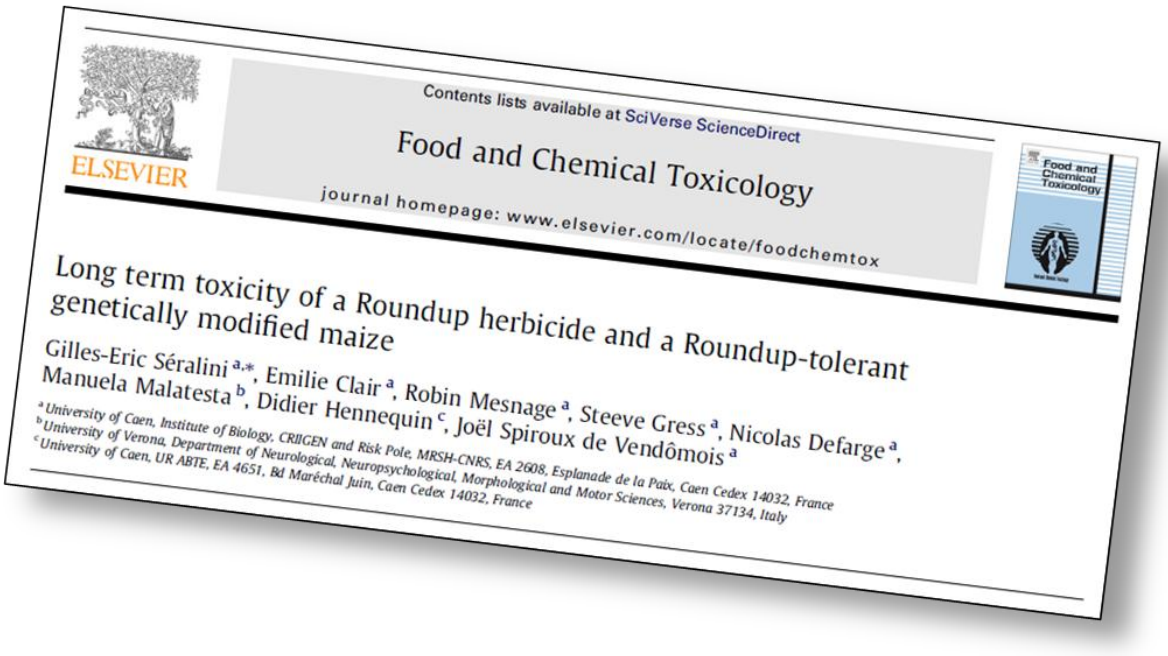
A scientific analysis of the rat study conducted by

Gilles-Eric Séralini et al.



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Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize

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Synopsis

On 19 September 2012, Gilles-Eric Séralini and his colleagues published a sensational study that, in their opinion, brought to the fore clear indications that genetically modified crops and Roundup are dangerous to health. Media across the world picked up on this report and published disturbing photos of rats with enormous tumors. Scientists reacted with shock and were quick to criticize the study. The scientific analysis in this document shows the research design that Séralini et al. used contained fundamental shortcomings that preclude any sensible conclusions from being drawn. In other words, the statements that Séralini made about the health effects of GMOs and Roundup were baseless. Moreover, the research shows signs of selective interpretation of the findings or a misleading representation of these, which is contrary to prevailing scientific ethical standards.

1. Background

On Wednesday, 19 September 2012, the scientific journal *Food and Chemical Toxicology* published online a study by Gilles-Eric Séralini and his team about the alleged effects of genetically modified maize and the herbicide, Roundup, on the health of rats.[1] In Séralini's opinion, the study showed alarming results. He sent shocking images around the world of rats with large tumors, images eagerly reproduced by the media. Sharp criticism of the study soon followed. Various scientists pointed to the significant shortcomings in the research and raised several questions. Among other things, a petition was launched requesting Séralini to release the data underpinning the study.

The Flemish Interuniversity for Biotechnology [*Vlaams Interuniversitair Instituut voor Biotechnologie* (VIB)] was also shocked at the images and the messages that the study provoked in the media. Let it be clear that VIB is in no way involved in the development of NK603[2] maize that the study used, and that they have no stake whatsoever in the herbicide Roundup. However, VIB is a world authority in plant research that uses genetically modified plants as a research resource. New knowledge that VIB garners in this way can, in some cases, contribute to the development of genetically modified crops. For this reason, VIB considers it their social duty to thoroughly examine new information about the possible health effects of genetically modified plants.

For this reason, a scientific analysis is given below of Séralini's study in which we would like to formulate an unequivocal answer to the question **of whether the study in question gave substantiated indications of negative effects on health as a consequence of consuming genetically modified food: YES or NO.**

2. The research design of the rat study conducted by Gilles-Eric Séralini et al.

Gilles-Eric Séralini and a team of colleagues wanted to investigate whether the continuous consumption of a certain genetically modified maize variety and/or the herbicide Roundup was damaging to health. To answer this question, they set up a **two-year long feeding experiment** with rats. Two years is the average lifespan of a rat.
Séralini fed the rats the following test diets:

1. A diet that partially comprised genetically modified NK603 maize in which he tested three different proportions: **11%, 22% and 33% genetically modified maize**. The remaining 89, 78 and 67% of the diet comprised standard, commercially available laboratory rat food made by the firm Safe.

2. A diet that, similar to the first diet, comprised in part genetically modified NK603 maize, but which had been sprayed in the field with the herbicide **Roundup**. Once again he tested three

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

[2] NK603 is a genetically modified maize variety, developed by Monsanto, that is resistant to the effects of the herbicide Roundup. Roundup is a so-called broad-spectrum herbicide that in principle kills all plants. Genetically modified NK603 maize has a property that makes it insensitive to Roundup, which means that Roundup can be sprayed on this maize to kill all weeds without being harmful to the maize itself.

different proportions: **11%, 22% and 33% genetically modified maize**. Whether there were any traces of Roundup on the maize that was fed to the rats, and, if so, how much, we do not know because it was not measured.

3. A diet that contained no genetically modified maize, but in which the rats had free access to drinking water that contained concentrations of the herbicide Roundup: **1.1 x 10-8%, 0,09% or 0,5% Roundup**.
   NB: There is a significant difference in concentration from the 1st to the 2nd sample (a difference of a factor 107).

4. A diet that contained **non-genetically modified maize** which had a genetic background that largely resembled NK603 maize. Thirty-three percent of this diet consisted of this maize and the remaining 67% comprised commercially available laboratory rat food.

Séralini et al. fed each of the test diets to a group of male and a group of female animals. In this way, they tested a total of 10 different diets, of which one was a control. For each diet, they tested 10 animals of each sex, or **200 rats in total**. All the animals had free access to the food and drinking water. In other words, there was no restriction on how many calories the animals could consume.

3. Breed of rats used

For their study, Séralini et al. used 'Sprague-Dawley rats'. This is a laboratory strain that is known for its propensity to the spontaneous development of tumors (see www.harlaneurope.com under 'lifespan and disease'). The number of tumors that the rats develop spontaneously depends on the amount of food that they are given[3]. The number of spontaneous tumors is highest when these rats are allowed to consume calories without any restriction[4]. Various figures for spontaneous tumors are reported. There are publications that mention 42 to 72% among female animals[5-6]. Among male animals these figures are slightly lower, but there is a study that mentions over 86%[7]. **The fact that these animals have a propensity for the spontaneous development of tumors was not mentioned by Séralini et al. in their article, nor how they addressed this in their research design**. The rats can also develop tumors at a very early stage. Schardein et al. (1968)[8] reported that 6 out of 3,000 rats spontaneously develop tumors within three months, 10 out of 700 within 6 months, 20 out of 400 within 9 months, etc., increasing within 18 months to figures approaching the percentages mentioned above for 2 years.

3 Davis R. K., Stevenson G. T. and Busch K. A. (1956). Tumor incidence in normal Sprague-Dawley female rats. Cancer Res 16:194-197.

4 Keenan K. P., Soper K. A., Smith P. F., Ballam G. C. and Clark R. L. (1995). Diet, overfeeding, and moderate dietary restriction in control Sprague-Dawley rats: I. Effects on spontaneous neoplasms.

Toxicol Pathol 23: 269-286.

5 Prejean J. D., Peckham J. C. and Casey A. E., Griswold D. P., Weisburger E. K. and Weisburger J. H. (1973). Spontaneous Tumors in Sprague-Dawley Rats and Swiss Mice. Cancer Res 33:2768-2773.

6 Kaspareit J. and Rittinghausen S. (1999) Spontaneous neoplastic lesions in Harlan Sprague-Dawley rats. Exp Toxic Pathol 51: 105-107.

7 Suzuki H., Mohr U. and Kimmerle G. (1979). Spontaneous endocrine tumors in Sprague-Dawley rats. J Cancer Res Clin Oncol 95:187-196.

8 Schardein J.L., Fitzgerald J.E., Kaump D.H. (1968). Spontaneous tumors in Holtzman-source rats of various ages. Pathol Vet 5:238-252.

4. Comments on the research design

Sprague-Dawley rats

It is not unusual to use Sprague-Dawley rats for food experiments. For instance, numerous food safety studies have been carried out on various genetically modified crops whereby the crops were fed to these rats over a period of 90 days. These rats are also often used to test the safety of chemical substances in similar 90-day food experiments. The OESO has developed guidelines for the way in which these experiments should be conducted (OECD guidelines for the testing of chemicals; health effects; test no. 408 [9]; Repeated Dose 90-day Oral Toxicity Study in Rats). In 90-day food experiments, the fact that the rats have a propensity to develop spontaneous tumors plays a minor role. This, however, changes if you use them to carry out two-year studies. Then it plays a much more significant role, because how, in such a long-term experiment, can you differentiate between tumors that occur spontaneously from those that occur **as a consequence of** eating genetically modified maize, or drinking Roundup. Experts in the fields of toxicology, food experiments and statistics indicate very clearly that the number of animals per group in this case must be raised drastically, to a minimum of 50 or even 70 per group. With only 10 animals per group you cannot claim with any certainty whether a tumor developed spontaneously or is a tumor that developed as a consequence of a specific diet.

The number of control groups and the number of control animals

In their experiment, Séralini et al. not only use too few animals per group, they also use only 10 control animals (1 group) per sex compared to 90 treated animals. This control group was given a diet containing a 33% proportion of non-genetically modified maize, the remaining 67% being standard laboratory rat food. The same control group was used as a reference for all the treatment combinations. In this way, the control animals are less representative for the natural variations mentioned in Section 3 that are present in the population. On the basis of simple probability calculations, it can be concluded that the chances of finding spontaneous tumors in the group of treated animals is much greater than the chances of finding spontaneous tumors in the control group. **This is a fundamental error in the research design: there are too few control groups in relation to the treated groups.**

At the very least, there should have been a control group for each type of treatment: a control group for 'GMO maize treatment', a control group for 'GMO maize + Roundup treatment', and a control group for 'Roundup treatment', and then each of these by sex. Other negative checks that would have improved the quality of the experiment include:

- 100% standard laboratory rat food.
- A few non-genetically modified maize varieties that had a genetic background which differed from NK603, that in some way would be representative of maize varieties that are commonly grown.

When a laboratory does not have statistics relating to spontaneous tumor incidence in the circumstances being applied, the control group used in the experiment is in most cases much larger, often twice as large as the group being treated. This is how one attains a correct internal standard for spontaneous tumors. This is done because the incidence of spontaneous tumors is strongly influenced by the experimental circumstances, by dissection and tissue preparation for histopathology, and by the criteria used to identify and categorize the tumors.

9 http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

The purpose of controls in a scientific experiment
A good scientific experiment always has sufficient negative controls and, if available, positive controls as well. A negative control is a treatment that you expect or know will have no effect. A positive control is a treatment that you know beforehand is bound to have a significant effect, and preferably you also know how large the effect will be. Positive controls are hardly, if ever, used for cancer studies. The controls are included in the experiment to assess whether it went well. If the negative control has an effect then you know that something went wrong in your experiment. Perhaps you forgot to include something, maybe the equipment failed in some way, or the samples were contaminated. In any event it is sufficient reason to disregard the results and start the experiment again from scratch. Every critical analysis of the results of scientific experiments starts with an analysis of the controls.

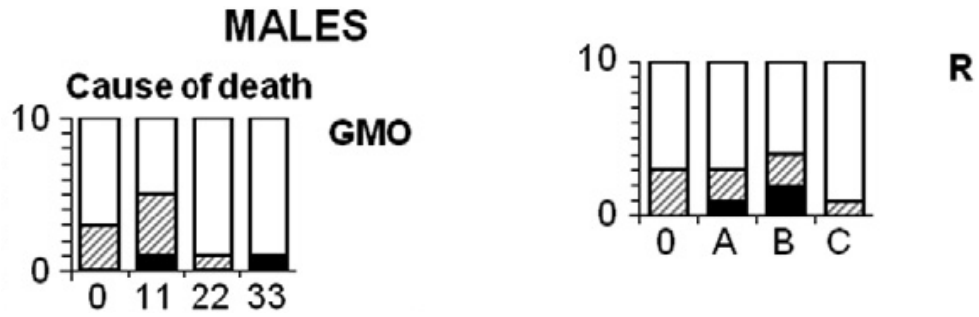
What is the significance of the fact that only 10 animals were tested per treatment?
Let's conduct the experiment in concrete terms. We start with 200 randomly chosen young Sprague-Dawley rats, 100 males and 100 females which, at the start of the experiment, must be divided into groups of 10. If we assume that under the circumstances that we subject them to, regardless of any specific treatment, 60% of the females and 50% of the males will spontaneously develop tumors, it means that we must divide the animals into groups of 10 that among the females six in each group will develop tumors spontaneously and among the males this will be five. We do not know beforehand, however, which of these animals will develop tumors spontaneously. The chances that we will have made groups of 10 among the females in which in the one instance two and in the other instance nine animals will spontaneously develop tumors, or four and eight instead of six, are extremely good. Only once you have increased the size of the groups significantly will the chances that you have divided the animals incorrectly drop considerably. **This is the second fundamental flaw in the research design used by Séralini et al. They use far too few animals per treated group**.

'New research shows that Dutch people are more than twice as likely to have blue eyes than Flemish people'
A researcher selects ten randomly chosen Flemish people and ten randomly chosen Dutch people, and objectively observes that, among the Flemish, three people have blue eyes, and among the Dutch, seven. He goes on to draw the conclusion that the Dutch are more than twice as likely to have blue eyes than the Flemish. But is his conclusion correct? We put the evidence to the test and repeat the experiment. We go on to find six Flemish people that have blue eyes and four Dutch people with blue eyes. What does this mean? This means that he did not make enough observations to achieve a repeatable experiment and, as such, to achieve relevant results, and that the researcher's conclusion was premature. Sample sizes and the number of repetitions are extremely important if you intend to make statements with any degree of certainty about things of this nature. This is why the statistical principles of any scientific experiment must be given for the sample sizes chosen, and why aspects such as variance and standard deviation must always be mentioned.

5. The results of the experiment

Despite the abovementioned fundamental errors in the research design, we will subject the results to further scrutiny. What do we discover?

Negative checks in comparison with the treatments
The results of Séralini et al.'s experiment show that there were **fewer deaths among the male animals whose diet comprised food with 22 or 33% genetically modified maize (= negative control)** (the left hand side of the figure below). This is remarkable given that the genetically modified maize was herbicide tolerant, and no new properties that may have had health advantages for the rats. We see the same result after Roundup was added to the drinking water. There were fewer deaths among the male rats that had drunk the highest concentration of Roundup than among those who had drunk pure water (the right hand side of the figure below). And this while Roundup certainly does not contain any known life-extending properties. The researchers should have taken these observations as a warning that there was something wrong with the experiment, because if these results were correct it would mean that consuming large amounts of genetically modified NK603 maize or Roundup would be a way to live longer. These strange findings are not interpretable because as noted previously **there is something fundamentally wrong with the research design.**



11 = 11% NK603 in the food
22 = 22% NK603 in the food
33 = 33% NK603 in the food

A = 1.1 x 10-8% Roundup in the drinking water
B = 0,09% Roundup in the drinking water
C = 0,5% Roundup in the drinking water

Figure 1. **The number of animals that died during the experiment is lower in the groups that ate genetically modified NK603 maize (left hand figure) and drank Roundup (right hand figure).** *The shaded area illustrates how many animals died during the experiment, and the black area within this indicates the number of animals that needed to be put down to end their suffering.*

Gilles-Eric Séralini's background
Gilles-Eric Séralini works at the University of Caen in France. He works there in the Oestrogen and Reproduction Laboratory of the Biology Institute and is co-director of the Risks Pole-Quality and Sustainable Environment – MRSH-CNRS [*Pôle Risques, Qualité et Environnement Durable - MRSH-CNRS*].

Séralini is co-founder of CRIIGEN, the 'Committee for Research & Independent Information on Genetic Engineering' (www.criigen.org). This is an organization that has an extremely negative opinion when it comes to genetically modified crops and is undertaking an active campaign against them.

This is not the first time that Séralini and his team have presented research findings that, according to them, indicate the potentially harmful effects of genetically modified organisms. Scientists and official advisory organizations, such as EFSA (European Food Safety Authority), consigned all these earlier studies to the bin because there was no scientific underpinning for the conclusions that they presented.

Analysis of the death rate
There is no statistical analysis of the number of deaths in the manuscript. Based on the figures, there are virtually no reliable differences in the death rates to be found among the males. This is probably because the average death rate within the control group is virtually the same as the average of all the treated groups put together. Among the female animals, there were differences, but this is probably because the average death rate in the control group is low in comparison to the treated group, but is also half as low as it is for the male animals.

The research design once again plays a role in the analysis. Because there are not enough control groups and control animals in the study, there is also insufficient reliable data on spontaneous death. If Séralini et al. had included a control group for each treatment, or a control group that was twice as large as the treated group to compensate for the lack of internal standards, then they would most probably have found a variance in death rate among the control group that they have now avoided. It is not clear whether all the animals died of tumors.

The conclusion is that **Séralini et al. did not find any reliable differences in the death rates between the treated and untreated animals**.

Analysis of the tumors
Séralini et al. make an unusual distinction between small and large tumors and between external palpable tumors and internal tumors. In carcinogenicity studies, tumors are normally always investigated separately: the incidence per type of tumor is looked at and compared with the incidence in the control animals, and with the historical incidence in the lab itself (which is lacking in this study).

Séralini et al. find differences in the number of large tumors; in the differences in the number between the males and the females in the control group; and a difference in the number of large tumors among the treated and control female animals. The size of a tumor, however, is not related, on the face of it, to how serious it is. Séralini et al. had to put down all the female control animals that developed tumors before the end of the experiment to put an end to their suffering, which is a measure of the seriousness of the tumors.

The fundamental problems in the research design mentioned in Section 3, in particular the chances of dividing the animals incorrectly when grouping them and the imbalance between the number of animals in the control group and the treated animals, **do not allow any statistically underpinned conclusions to be drawn in relation to the tumor formation**.

Dose-effect relationship

Further analysis of the data in the study shows that **no relationship was found between the dose** (the amount of GMO and/or Roundup) and the effect (tumors/pathologies/death rate). Séralini et al. acknowledge this in their article and explain it by claiming that:

> "*As is often the case for hormonal diseases, most observed effects in this study were not proportional to the dose of the treatment (GM maize with and without Roundup application, Roundup alone), non-monotonic, and with a threshold effect.*"

What they fail to report, however, is that the observed effects in many cases overlap with the effects that were observed in the control groups. **They can only invoke non-dose-related effects as an explanation if these effects are not observed in the control group, and that is not the case.** Over and above this, in their conclusion Séralini and his team attribute the non-dose-related effects to the non-linear endocrine-disrupting effects of Roundup. They ignore the fact that comparable non-linear effects can also be seen in the treatments that did not include Roundup, perhaps because this would undermine their conclusion. And, as we noted previously, for several of the treatments the lowest mortality was among those who had been given the highest doses. Mortality increases in line with the dose when the substance is in actual fact carcinogenic.

Because of the small number of control animals and the absence of adequate controls, the reliability of the limited data is seriously compromised and so Séralini et al. go to great lengths to find explanations for their findings. **They ignore, however, the most obvious explanation, namely that the established variability in the data is not supported by a proper research design, which precludes adequate interpretation of the data**. Moreover, they use an unorthodox statistical method ('two class discriminant analysis') that aims at finding differences instead of investigating differences between the treated animals and the control group.

In other words, they are only looking for interpretations that support their theory.

Misleading

There are also other places in the publication where there is evidence of incorrect interpretation of the results or a one-sided presentation of these. For instance, there is only a photograph of a treated rat that developed a tumor. There are no photographs of control rats. It was this photo of the rat that was sent around the world. And, to show the pathologies that developed in greater detail, rats from the control group that had not developed tumors were selected, while from the treated group rats were selected that had developed tumors. On the basis of previous publications as well as from data from Séralini's study, we know that rats in control groups also develop tumors.

6. Other shortcomings

Alongside the issues described in the sections above, several scientists have pointed out all kinds of other shortcomings and have listed various concerns, including:

- The experiment does not meet the OECD guidelines for carcinogenicity tests in rats.
- There is no data about the quality of the maize that was used. When grown in damp conditions, maize can be plagued by fungi that can produce mycotoxins which are known to be hepatotoxic, toxic to the kidneys, carcinogenic and that can mimic the effects of estrogen.
- There is no mention of how the nutritional balance was kept in the various diets. If you replace 22 or 33% of the food with maize, you change the percentage or the amount of carbohydrates, protein, fats, fiber, vitamins and so on, in the diet. You must then correct for this to avoid measuring effects that are a consequence of changes in the nutritional balance.
- There is no data about the body weights of the animals, increase in body weight, and a discussion of this.
- There is no indication whatsoever of whether the genetically modified maize originating from the land that was sprayed with Roundup contained traces of the degradation products of Roundup and, if so, how much there was, and how this compared to the amount of Roundup that was fed to all the animals. Moreover, Roundup consists of various components: the active ingredient, glyphosate, and a few other substances (among others a substance that promotes distribution of the spray over the leaves). These various components and their degradation products may have been on the treated maize that was fed to the animals in completely different proportions than in 'pure' Roundup. Some break down very quickly. In other words, Roundup in the 'Roundup' treatment could be completely different to Roundup in the 'GMO maize + Roundup'.
- The type of statistics that was used is never used in the interpretation of tumor data.
- It is not clear whether the NK603 maize that was sprayed with Roundup was also treated with other herbicides and, if so, which.
- This study does not contain any appropriate statistical analysis of mortality, tumor incidence and general pathological findings. Had this been done, what would have emerged is what we indicated in Section 4: that, among other things, there is no significant difference in mortality.

7. Conclusion

The two-year long rat study conducted by Séralini and his colleagues displays, from a scientific point of view, considerable shortcomings. The most serious of these can be found in the fact that the study used far too few rats per treated group and that there were too few control groups. In one fell swoop this entirely removes the basis for the conclusions that Séralini et al. draw. In addition to this, for every conclusion that they draw there is sufficient evidence in their own text to undermine them completely. There are also other shortcomings and numerous other questions that remain unanswered. One thing is clear: **Séralini et al. have not been able to substantiate in any way whether genetically modified NK603 maize or Roundup is harmful or not**. The only thing that the study confirms is that Sprague-Dawley rats, like many other laboratory rats, develop relatively speaking many pathologies and that, as a consequence of this, many of the animals do not reach two years of age. But we have known this since the 1960s.

Epilogue: Analysis of a media campaign

Séralini used the online publication of his scientific article to start a large-scale media campaign. A press invitation was sent out and a press conference was organized in the European Parliament building under the auspices of European Member of Parliament, Corinne Lepage. Lepage was one of the founders of CRIIGEN and has been honorary chairperson of CRIIGEN since June 2012.

Journalists who wanted to read the article before the embargo passed had to sign a statement that they would not put forward the article to experts for their opinion before the passing of the embargo, which is in contravention of the deontological code of the journalistic profession. Journalists must always reserve the right to ask for a second opinion. This is how Séralini ensured that the first bulletins about their work were exclusively sensationalist reports about large tumors in rats as a consequence of eating GMO maize and/or drinking Roundup. Critical voices about the quality of their study generally followed in second place.

During the press conference, Séralini and Lepage did not restrict their statements to NK603 maize and Roundup. All genetically modified crops were depicted as harmful to humankind. The press conference was also seized on as an opportunity to announce the upcoming publication of a book and the release of a film. The intention was clearly not to elucidate the publication of a scientific article. All the signs pointed to a purposive, broader campaign aimed at putting genetically modified organisms in a bad light.