Feeding study in rats with genetically modified NK603 maize and with a glyphosate containing formulation (Roundup) published by Séralini et al. (2012)

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In mid-September 2012, a scientific team headed by Gilles-Eric Séralini at the University of Caen in France published the results of a long-term study with rats which had been fed genetically modified glyphosate-tolerant NK603 maize. One part of the maize had been treated with a glyphosate-containing plant protection product (*Roundup*) during cultivation, whereas another part was untreated. In each case the maize was administered in three doses. In addition, other animals fed with conventional feed received *Roundup* via the drinking water, also in three doses. The only control group was fed a non-genetically modified maize. The authors reported that the animals in some of the test groups developed increased incidences of several tumours and other non-neoplastic lesions and died earlier than animals in the control group. The effects could have been caused by hormonal effects of *Roundup* and specific constituents of the genetically modified maize, respectively. The Federal Institute for Risk Assessment (BfR) has evaluated the study in terms of its relevance for the evaluation of the health risk of genetically modified glyphosate-tolerant maize NK603 and also with regard to the evaluation of the health risk of the glyphosate-containing formulation. On the basis of the publication, the BfR has come to the conclusion that the authors' main statements are not sufficiently corroborated by experimental evidence, due to deficiencies in the study design and in the presentation and interpretation of the study results. Therefore, the main conclusions of the authors are not supported by the presented incomplete data. The study does not comply with internationally recognised standards for long-term carcinogenicity studies. The rat strain used shows a relatively high spontaneous tumour rate, especially for mammary and pituitary tumours, and the number of animals used was too small and insufficient for assessing the claimed differences between the test groups and the control group. Also the authors' hypothesis that the observed effects could result from adverse effects on the endocrine system is not sufficiently supported by the data presented. Furthermore, the BfR criticises that the glyphosate dose administered was not determined in the studies with the glyphosate-containing plant protection product *Roundup*. Due to these deficiencies the BfR has asked the authors to provide the complete study report including the individual animal data. Moreover, it has asked specific questions in order to allow for a further evaluation of the reported effects.

1 Subject

This opinion refers to the study "*Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize*" published by Séralini and co-authors in the journal *Food and Chemical Toxicology* on 19 September 2012 (Séralini et al., 2012).

2 Result

After having reviewed the publication, the German Federal Institute for Risk Assessment (BfR) is of the opinion that the experimental data do not support the main statements in the publication. Further, due to shortcomings in the study design as well as in the presentation and interpretation of the data, relevant conclusions drawn by the authors are not comprehensible.

For further assessment, the BfR has asked the authors to provide the complete study report including the individual animal data and has also put forward specific questions. This request has not yet been answered.

3 Justification

Based on scientific opinions of the European Food Safety Authority (EFSA) (EFSA, 2003a; EFSA, 2003b), the NK603 maize that was used in the study was authorised for feed use in accordance with Directive 2001/18/EC on 19 July 2004 and for food use in accordance with Regulation (EC) No 258/97 on 10 October 2004. An application for renewal of these authorisations in accordance with Regulation (EC) No 1829/2003 was already assessed by EFSA (EFSA, 2009).

The active ingredient in the *Roundup* formulation used in the study was glyphosate which was included in Annex I of Directive 91/414/EEC in 2002 for a ten-year period based on Directive 2001/99/EG from 20.11.2001 (European Commission, 2002). The submitted data were evaluated in the monograph (Draft Assessment Report, DAR) by Germany as Rapporteur Member State (RMS) with the involvement of the BgVV, the predecessor of the BfR, in 1998. This comprehensive assessment in the DAR has been supplemented several times. Following a decision of the European Commission, the inclusion of glyphosate in Annex I was prolonged until 31 December 2015 with Directive 2010/77/EC from 10 November 2010. Currently, a renewal of the assessment of glyphosate is ongoing within the AIR2 programme based on Regulation (EC) No 1141/2010. Germany acts again as RMS and will, with involvement of the BfR (responsible for drafting the chapters on toxicology, residues and analytical methods), establish a new DAR that will be discussed in the framework of the Community centralised procedure led by EFSA. Numerous glyphosate containing plant protection products have been authorised in EU countries (currently 75 herbicides in Germany including 13 different *Roundup* formulations).

Beside studies on potential health effects from genetically modified NK603 maize the researchers led by Gilles-Eric Séralini had published a series of papers on effects of glyphosate containing plant protection products. Some of them (Richard et al., 2005; Benachour et al., 2007; Bellé et al., 2007; Gasnier et al., 2009; Benachour and Séralini, 2009) had been already commented by the BfR.

The aim of the now published study by Séralini and co-authors was to examine potential effects of the genetically modified glyphosate-tolerant NK603 maize and of one glyphosate containing formulation (*Roundup*) administered to rats over two years. Groups of 10 male and 10 female Sprague Dawley rats (Harlan) were fed diets containing 11, 22 or 33 per cent of NK603 maize (Monsanto Corporation, USA), which had been treated or not treated with *Roundup* during cultivation. The diet for the control group contained 33 per cent of a non-genetically modified maize line. The animals of another test series received drinking water containing 1.1×10^{-8}, 0,09 or 0,5 per cent *Roundup* .

The authors concluded from the results of the study that the mortality of female animals in all treated groups as well as male animals in three of the groups that had received NK603 maize was higher than in the control group and deaths occurred earlier. According to the authors, all results were hormone and sex dependent, and the pathological profiles were comparable. It was postulated that females developed more frequently large tumours of the mammary gland, and the pituitary was the second most affected organ. In treated males, pathologic lesions in the liver (congestions and necrosis) and kidney (severe nephropathies) were more frequent, the latter was confirmed by biochemical data. The authors further indicated that these results could be explained by non-linear endocrine-disrupting effects of *Roundup*, but also by overexpression of the transgene in the NK603 maize and its metabolic consequences.

BfR noticed with interest that for the first time a long-term feeding study was performed with a glyphosate containing formulation. Long-term studies were not yet available because for regulatory purposes such studies are worldwide requested only with the active substances. Glyphosate itself has been comprehensively tested. Numerous long-term studies in rats and mice showed no indications of either a carcinogenic potential or increased mortality or any effects on the endocrine system, as reported by Séralini and co-workers in their publication.

However, the BfR is aware of certain co-formulants, in particular surfactants from the group of polyethoxylated alkyl amines (POEA, often designated as tallow amines), that might affect the toxicity of glyphosate containing herbicides. The toxic effects are in some cases more severe compared to studies with the active ingredient. Therefore, the results of the study performed by Séralini's group could provide an experimental contribution to the elucidation of the possible influence of formulants on long-term effects of plant protection products.

While the performance of a long-term study in the case of the glyphosate containing formulation is in principle appreciated, it needs to be mentioned that the published study shows significant shortcomings in the study design and further shortcomings due to incomplete and unclear presentation of the collected data. Furthermore, the main statements were not supported by the experimental data. As outlined in detail below, it is therefore impossible to comprehend the main conclusions of the authors.

3.1 Comments on the study design

Long-term studies are highly complex and elaborate as rats spontaneously develop tumours and other age-related alterations. The published study was not conducted in accordance with internationally accepted standards, such as OECD Test Guidelines No. 451 or Nr. 453 (OECD, 2009a; OECD, 2009b). Instead, the authors have chosen a study design (OECD Test Guideline Nr. 408) that was developed for 90 day (subchronic toxicity) studies (OECD, 1998). Therefore, only 10 animals per sex instead of 50 have been assigned to each group.

However, subchronic studies show a substantially lower variation of age-related pathological changes between animals within a group while those changes are inevitable in long-term studies. As the published study has confirmed, the two-year duration of the study is of the order of the expected life span in rats including the Sprague Dawley strain that was used in the study. This strain, provided by the breeder Harlan, is known to develop spontaneous tumours, particularly mammary and pituitary tumours, at relatively high rates compared to other strains (Brix et al., 2005; Dinse et al., 2010). Therefore, it can be expected that a significant number of animals develop age-related illnesses or die for diverse reasons already during conduct of the study. The distribution of the cases of death between groups can be random, and a number of 10 animals per sex and group is too low to confirm a trend or an effect. Furthermore, no statements on statistically significant dose-response-relationships can be made. Larger sample sizes, as recommended for carcinogenicity studies in OECD Test Guidelines No. 451 or No. 453, would be required in order to allow precise statements with respect to the findings.

Regarding the design of the study, another point of criticism is that the mean levels of the daily applied doses of *Roundup* have not been determined. It should also be noted that the glyphosate containing formulation (Weather-MAX) used for the treatment of NK603 maize during cultivation was different from the formulation (GT Plus) used in the test series with *Roundup*. Further details on the composition of the applied formulations are lacking.

The publication does not inform whether the diets of all groups contained a total of 33 per cent maize, i.e. whether the diets with 11 and 22 per cent have been supplemented with non-genetically modified maize. The only information given by the authors is that balanced diets were fed and that these diets were considered "substantially equivalent" except for the newly introduced gene. However, detailed information on the composition of the diets is lacking. Moreover, data on feed and water consumption as well as body weight development are missing. The question therefore is, whether balanced diets really had been administered. There are also no further details on the identity of the control maize line that is referred to as "nearest isogenic non-transgenic control". Furthermore, it has to be critically stated that the maize varieties used in the study were not analysed for the presence of mycotoxins.

3.2 Comments on the presentation of results

The first part of the study considers mortality, tumour incidences and other pathological changes and contains descriptive data while statistical analyses are lacking. The presentation of the data in percentage terms or as “x times more”, suggest more impressive results compared to absolute figures.

The BfR is of the opinion that the treatment-related increase in mortality as reported by the authors is not confirmed by the published data. The two cases of death caused by Wilms’ tumours (nephroblastoma) in male animals of two not clearly indicated test groups fed with *Roundup* treated NK603 maize are not chemically induced and are correctly not claimed to be treatment-induced. Therefore, they should not be used as evidence for a higher mortality compared to the non treated control group. Likewise, no effects of *Roundup* on the mortality of male rats can be detected.

In female rats mammary tumours are indicated as the main cause of mortality. However, this type of tumour occurs rather frequently particularly in Sprague Dawley rats and if feed is offered *ad libitum*. In the current study this type of tumour also occurred in approximately 50 per cent of the animals in the control group. As outlined above, the number of animals is not sufficient for an assessment of the difference to the treated animals (60 to 100 per cent without a clear dose dependence). The reported comparison with historical control data published in 1992 is not acceptable.

The incomplete and undifferentiated presentation of the data makes evaluation very difficult. For example, it is absolutely insufficient to mention only findings in liver and the digestive tract, as done in table 2, without characterising them from a differential diagnostic standpoint and assessing the grade of severity. Further, the graphs demonstrating mortality and tumours, respectively, are not always in agreement with the statements in the text or can not be followed, as in the case of the observed deaths caused by Wilms' tumours.

A statistical analysis was only performed for the biochemical parameters. This was done with a special kind of principal component analysis (OPLS-DA = Orthogonal Partial Least Squares Discriminant Analysis), but results were only presented for one group (females that had received feed with 33 per cent NK603 maize compared to the control group). In addition, figure 5, presenting biochemical parameters, is difficult to understand. Their assessment would require data of all measurement time points.

3.3 Comments on the mechanisms suspected by the authors

One hypothesis of the authors was that specific compound(s) present in the genetically modified NK603 maize and in the applied glyphosate containing formulation, respectively, could

account for the observed increased tumour incidences, particularly in female test animals, by affecting the endocrine system. However, the BfR is of the opinion that no convincing arguments have been provided to support this. The following points are discussed.

- The authors indicate that most of the observed effects show a non-monotonic dose-response-relationship and show a threshold. They consider this as a clear indication that the endocrine system is adversely affected. The authors refer to a recent review published by Vandenberg et al. (2012). However, a detailed look into this paper reveals that its content is not correctly reflected by Séralini et al.. Vandenberg et al. explicitly question the existence of a threshold for adverse effects induced by endocrine disruptors. Thus the cited literature is not suitable to support the authors' claims. Furthermore, the presence of a non-monotonic dose-response- relationship does not mean that the effects are caused by an impairment of the endocrine system. Non-monotonic dose- response-relationships have also been described for other substances. For example some essential minerals show a non-monotonic dose-response-relationship (Stern, 2010; Calabrese, 2008) yet without affecting the endocrine system. Considering a non-monotonic dose-response-relationship, a quantitative relationship between the dose and the effect is observed which, however does not proceed in a monotonic manner over the examined dose range. Instead of a non-monotonic dose-response-relationship, the data presented does not allow the identification of any obvious relationship between the observed adverse effects and the applied dose. Rather, the datasets consisting of 3 dose levels and the control group with animal numbers <10 per group and sex show no statistically significant relationship between the observed effects and the applied dose.

- To further support their thesis the authors refer to their results obtained by measurement of testosterone and estradiol levels (figure 5B). The figure presents the data for hormone levels of the single female animals of the control group and the group, which had received a diet with 33 per cent NK603 maize, 15 months after the commencement of the study. A balanced scientific discussion should include a critical discussion of specific points by the authors. For example, statistically significant differences in hormone levels might easily be assessed on the basis of mean values plus standard deviation. However, figure 5B does not provide a clear basis to perform a statistical evaluation with sufficient accuracy. In addition, the respective data for male animals were not shown. Furthermore, the natural variation in hormone levels caused by the circadian rhythmic and during the estrous cycle was not acknowledged by the authors as a possible cause for the results given in figure 5. It is also known that Sprague Dawley rats develop estrous cycle abnormalities relatively early (from 4-6 months of age; OECD, 2009). The differences observed between treated and control animals 15 months after study begin could thus also be due to variations in hormone levels independent of the applied substances. If the authors were right in stating that the particularly higher incidence of mammary tumours could be related to the estradiol level, one would expect a statistically significant difference in the estradiol level of the female animals in the group, which had received a diet with 33 per cent NK603 maize, when compared to the control animals. However, this is not identifiable on the basis of the data presented.

- The authors also hypothesise that NK603 maize and *Roundup* could cause hormonal disturbances via an impact on the estrogen system. In this respect, the authors regard lower contents of specific organic acids (caffeic and ferulic acid) present in NK603 maize as being responsible for the observed effects. These acids are claimed to exert protective effects in the experimental animals and to impact on the estrogen

metabolism. However, significant differences in the estrogen levels of female animals in the group fed with 33 per cent NK603 maize can not be identified on the basis of the data presented. Additional factors, for example a possible modulation of the ER-receptor expression have not been addressed experimentally. Furthermore, the discussion of possible protective effects by plant constituents on tumour development does not reflect the current state of scientific knowledge. With regard to effects induced by the glyphosate containing formulation, the authors discuss the possibility of aromatase inhibition as well as an interaction with cellular estrogen or androgen receptors. However, these anticipated mechanisms have not been tested experimentally in this work. They are based on results from *in vitro* studies, which have been questioned by the BfR in previous opinions. The thesis of the authors that the observed effects could result from adverse effects on the endocrine system, exerted by the genetically modified NK603 maize and *Roundup*, respectively, are therefore not sufficiently supported by the experimental data presented in the publication.

4 References

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