

## **The Danish National Food Institute's assessment of a new long-term study of genetically-modified maize NK603 and the herbicide Roundup**

The National Food Institute is of the opinion that the new study has not been correctly designed, that it does not use the correct statistics and, finally, that the authors do not discuss their data as prescribed by scientific practice in the field of toxicology. The Institute concludes that the article is of poor scientific quality and should not have been published in a peer-review journal.

### **Introduction**

The new study referred to is described in the article by Séralini G, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, Spiroux de Vendômois J, 2012: 'Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize', which was accepted for publication in the journal Food and Chemical Toxicology.

The article describes results from a two-year feeding study of rats of the type Sprague-Dawley (SD). The objective was to show the effects on rats fed with different concentrations of genetically-modified maize NK603 and the effects on rats of adding Roundup to their drinking water. NK603 maize is genetically modified in order to tolerate glyphosate, which is the active substance in Roundup herbicide. The study covered both NK603 maize sprayed with Roundup and NK603 maize not sprayed with Roundup. NK603 maize is approved for consumption in the EU and many other countries.

A total of 200 SD rats were used for the study, 100 of each sex, divided into groups of ten. Three groups of each sex were fed with NK603 maize in concentrations of 11%, 22% and 33% respectively. Another three groups received three different concentrations of Roundup in their drinking water. The third set received three different concentrations (11%, 22% and 33%) of the genetically modified NK603 maize which had been sprayed with Roundup. The last group was the control group, whose diet contained neither GM maize nor Roundup.

The article attempts to show that more rats died in the groups which received NK603 maize or Roundup in their drinking water than in the control group. It also attempts to show that there was a higher rate of tumours and that the rats died earlier in the groups which received NK603 maize or Roundup in their drinking water than in the control group, which was fed with conventional maize and did not receive Roundup in its drinking water.

One of the National Food Institute's tasks is to perform risk assessments and disseminate results, also in the field of genetically modified foodstuffs. It is our assessment that the article does not meet normal scientific standards in this area and has thus given rise to unnecessary concern about the effects of genetically modified maize and the herbicide Roundup. We have therefore decided that it is important to correct any

misunderstandings by pointing that the article contains methodological errors which led to incorrect conclusions.

### **The National Food Institute's assessment**

The Institute has read the article carefully and identified a large number of problems which make it impossible to draw conclusions concerning effects of the genetically modified maize or Roundup. The data presented in the article do not provide a basis for changing previous assessments of either the genetically modified maize NK603 or the active substance glyphosate contained in Roundup.

The most important objections to the article are based on the fact that each group contains too few animals to demonstrate effects, and the study does not meet the internationally accepted guidelines for long-term studies. Basic statistics on the animal experiment are lacking, and the statistical methods used to document the biochemical effects observed were not applied in a manner consistent with the relevant OECD guidelines on statistical reporting.

The authors conclude that there is higher mortality and a higher rate of tumours in the treated animals, but mortality and the numbers and types of tumours in all test groups lie within the range seen in control groups in other trials using Sprague-Dawley rats.

The assessments presented by the authors concerning effects of NK603 maize and Roundup are not consistent with other studies in which NK603 maize or Roundup have been investigated.

The National Food Institute considers it ethically irresponsible to let animals suffer with tumours for so long without any contribution to obtaining important data.

The Institute is of the opinion that the article is of poor scientific quality and should not have been published in a peer-review journal.

The reasons for this assessment are based on various aspects of the trial design and the interpretation of results which ensue from the article and are described in more detail below.

### **Trial design**

- A research protocol which meets the OECD standard for this type of animal test is not used. Among other things, the number of animals (ten per group) is well below what is recommended. This means that major significance cannot be attached to the results, as small differences will be hidden in the large random variations.
- Normally, in this type of trial, the same number of animals are used in the control and test groups. In some cases, the same control group can be used for several test groups. In this trial, ten animals per group are used, and only one group for each sex is a control group, which is used for comparison with all nine test groups. This represents far too few animals in each group for a long-term study of this type. Firstly, it can be expected that, with ten animals per group, not enough of them will survive at the end of the trial for it to be possible to produce statistics. Secondly, the control group can easily deviate significantly for random reasons, without any possibility of confirmation one way or the other. In the latter case an abnormal control group can give rise to false indications suggesting that all test groups deviate from what is

normal. More control animals, combined with a reasonable statistical analysis, could be used to reduce uncertainty concerning assessment of whether the control group represents what is 'normal', and whether the findings reported in respect of the test groups can be linked to treatment. The choice of Sprague-Dawley (SD) rats was not a good one, as the species is known to be susceptible to spontaneous tumours (prevalence approx. 45%) and has a relatively high mortality rate (in two-year trials an average of less than 50% will survive<sup>1</sup>). The article does not give any justification for the choice of rat species.

- From the ethical point of view, the study gives rise to problems in several fields. The number of animals is so small that any effects cannot be identified through a statistical analysis of data. The authors themselves refer to an article<sup>1</sup> which describes survival rates for SD rats during long-term studies, which means they were already aware of the problem before starting the study. Another aspect is that they allowed tumours to grow very large before the animals were killed. There is no scientific reason not to kill animals with tumours earlier. We consider it ethically irresponsible to allow animals to live for so long with tumours, if this does not contribute to obtaining important data. The guidelines applied by the National Food Institute for animal testing ensure that tumours do not affect an animal's condition. Our criteria are that the weight of an individual tumour should not exceed 4 g, corresponding to a diameter of 20 mm, and that the overall tumour mass should not exceed 10% of the rat's normal weight. These criteria are consistent with the guidelines of the Animal Experiments Inspectorate (*Dyreforsøgstilsynet*) relating to authorisation to perform animal testing of this type.

It is not clear from the article how closely (genetically) the control maize is related to the genetically modified maize NK603. Nor are there any data on mycotoxin content or other cultivation aspects which may be of significance for the trial. Mycotoxins can be very damaging to the liver or kidneys, and may be carcinogenic, which is why the authors should have performed these measurements. It is not made clear whether all animal groups received the same concentration of maize (whether there was compensation with conventional maize in feed mixtures where, for example, 11% GM maize was given).

- There are no data on the animals' intake of food or water. These are significant parameters for assessing the animals' condition, e.g. it is conceivable that rats will avoid drinking or will drink less, simply because drinking water containing Roundup will have a taste which discourages them from drinking what they need.

## Results and conclusions

- The study consisted of a test series in which SD rats received different concentrations of Roundup in their drinking water. The active ingredient in Roundup is glyphosate. Glyphosate has been examined in many long-term studies in both rats and mice. None of these studies suggests that glyphosate has a potential to be carcinogenic, or that the animals have experienced increased mortality rates or hormonal effects. Apart from glyphosate, the herbicide Roundup contains various co-formulants, e.g. surfactants, but it is not clear from the article which substances are involved.

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<sup>1</sup> Brix, A.E., Nyska, A., Haseman, J.K., Sells, D.M., Jokinen, M.P., Walker, N.J., 2005. Incidences of selected lesions in control female Harlan Sprague-Dawley rats from two-year studies performed by the National Toxicology Program. *Toxicol. Pathol.* 33, 477–483.

The design of the study is so inadequate that it is not possible to decide whether or not adding Roundup to drinking water has an effect on rats in a long-term study.

The conclusions drawn by the authors on the basis of this material are therefore invalid.

- The only results presented by the authors from their comparative studies of the substances contained in NK603 maize and control maize are that ferulic acid and caffeic acid concentrations are much lower in NK603 maize — regardless of whether the maize had been treated with Roundup or not. The authors argue that these acids serve to protect animals, and that NK603 maize therefore lacks this protective effect, which can explain certain negative effects on the kidneys of animals fed with NK603. These differences have not been observed in previous studies in which genetically modified maize NK603 was investigated. The authors neglect to mention this. It can therefore be concluded that it is not the new protein, CP4-EPSPS, found in NK603 or other aspects related to the genetic modification of NK603 which is the cause of the lower amounts of ferulic acid and caffeic acid recorded in the study. Possible negative effects on the kidneys as a consequence of the low levels of ferulic acid and caffeic acid are therefore not related to the genetically modified maize, but to all types of maize containing low levels of these acids.
- There are no dose-response relationships for parameters reported in the study. A certain dose-response relationship would normally be expected, such that the animals fed with the largest amounts of a test substance show the most significant effects. In this study this relationship is not observed. The authors refer to the fact that this is not the case for all substances, but depends on the substance's effect on the animals. The National Food Institute is aware of these circumstances, but feels that the interpretation of the results obtained, including the fact that this can be put down to hormonal effects, is not substantiated.
- Conclusions are drawn from differences based on a small number of animals, which the authors believe can be considered significant. There is no neutral statistical analysis of data, e.g. comparison of mortality rates or numbers of tumours between the different groups.
- The data on mortality and occurrence of tumours provided by the study fall within the range of historical data for SD rats<sup>2</sup>, e.g. the survival rate (17-62.9% in males and 20-62% in females). The authors do not explain the frequency of animals with malignant and benign mammary tumours separately. The tumours found in the mammary gland tissue of female rats (fibroadenomas and adenocarcinomas) are known to be common in older female rats and their frequency is known to vary significantly between studies<sup>3</sup>.
- There is no balanced scientific discussion. Generally speaking, the authors fail to refer to relevant and important literature on the subject and do not compare their results with similar studies already published. They argue in the article that male rats die

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<sup>2</sup> Compilation of Spontaneous Neoplastic Lesions and Survival in Crl:CD® (SD) Rats from Control Groups. Charles River Laboratories March 2004.

<sup>3</sup> Greaves P, 2000: Mammary Gland and Haematopoietic and Lymphatic Systems In: Histopathology of Preclinical Toxicity Studies. Interpretation and Relevance in Drug Safety Evaluation. 2nd edition, pp 55-125, Elsevier Science.

mainly as a result of a significantly reduced liver and kidney function. However, these changes are part of the pathology of ageing rats, i.e. they are quite normal. This relationship to treatment must therefore be evaluated with great care, and a potential relationship with treatment should be claimed only if prevalence in the treated groups is significantly higher, in statistical terms, than in the control groups, and there is a clear relationship between dose and response. The article does not contain any data which can substantiate the statement, e.g. data from microscopic examinations of liver and kidneys, liver function measurements, urine analyses or cytochrome activity. In support of their arguments, the authors refer to their earlier publication<sup>4</sup>. However, this earlier publication, a statistical reanalysis of existing data, has previously come in for serious criticism by both experts and authorities for its lack of plausible scientific explanations.

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<sup>4</sup> Spiroux *et al.* Int. J. Biol. Sci. 5:706-726, 2009