STATEMENT OF EFSA

Review of the Séralini *et al.* (2012) publication on a 2-year rodent feeding study with glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology [1]

European Food Safety Authority[2, 3]

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ABSTRACT

On 19 September 2012, Séralini *et al.* published online in the scientific journal Food and Chemical Toxicology a publication describing a 2-year feeding study in rats investigating the health effects of genetically modified (GM) maize NK603 with and without Roundup WeatherMAX® and Roundup® GT Plus alone (both are glyphosate-containing plant protection products). EFSA was requested by the European Commission to review this publication and to identify whether clarifications are needed from the authors. EFSA notes that the Séralini *et al.* (2012) study has unclear objectives and is inadequately reported in the publication, with many key details of the design, conduct and analysis being omitted. Without such details it is impossible to give weight to the results. Conclusions cannot be drawn on the difference in tumour incidence between the treatment groups on the basis of the design, the analysis and the results as reported in the Séralini *et al.* (2012) publication. In particular, Séralini *et al.* (2012) draw conclusions on the incidence of tumours based on 10 rats per treatment per sex which is an insufficient number of animals to distinguish between specific treatment effects and chance occurrences of tumours in rats. Considering that the study as reported in the Séralini *et al.* (2012) publication is of inadequate design, analysis and reporting, EFSA finds that it is of insufficient scientific quality for safety assessment. Therefore EFSA, concludes that the Séralini *et al.* study as reported in the 2012 publication does not impact the ongoing re-evaluation of glyphosate, and does not see a need to reopen the existing safety evaluation of maize NK603 and its related stacks. EFSA will give the authors of the Séralini *et al.* (2012) publication the opportunity to provide further information on their study to EFSA.

Key words

Maize NK603, Roundup, glyphosate, experimental design, rat/rodent feeding study, toxicity, carcinogenicity

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 19 September 2012, an article[4] was published online in the scientific journal Food and Chemical Toxicology that described a 2-year rat feeding study investigating the health effects of genetically modified (GM) maize NK603 sprayed during growth with or without a Roundup® (glyphosate-containing plant protection product) and of Roundup® alone. The authors of the study conclude that low levels of glyphosate herbicide formulations, at concentrations well below officially set safe limits, induce severe hormone-dependent mammary, hepatic and kidney disturbances in rats. Similarly, they report disruption of biosynthetic pathways that may result from overexpression of the EPSPS transgene in the maize NK603. The authors suggest that such disruptions may have given rise to comparable pathologies that may be linked to abnormal or unbalanced phenolic acid metabolites or related compounds.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA received a mandate from DG SANCO on 26/09/2012 requesting to address the following terms of reference as a matter of urgency.

(A) Review the scientific publication

(B) Ask any clarification needed to the authors

(C) Advise whether the publication contains new scientific elements that could lead EFSA to reconsider the outcome of its opinion on maize NK603 and its related stacks

(D) Take into consideration the assessment of Member States

(E) Take into consideration the assessment of the German authorities responsible for the evaluation of glyphosate

EFSA'S APPROACH TO ADDRESS THE TERMS OF REFERENCE

EFSA decided to address the terms of reference (ToR) in phases. This first EFSA statement addresses ToR A, B and C solely based on the study information available through the Séralini *et al.* (2012) publication.

A second EFSA output will cover all the ToRs and will take into account any information received from the authors, the assessment activities from the Member States and the assessment of the German authorities responsible for the evaluation of glyphosate.

Following the publication of Séralini *et al.* (2012), EFSA set up an internal task force chaired by the Director of Regulated Products (REPRO) and composed of staff scientists with expertise in biostatistics, experimental design, mammalian toxicology, biotechnology, biochemistry, pesticide safety assessments and GMO safety assessments.

The task force was mandated to draft this EFSA statement which has been peer reviewed by two experts from EFSA's scientific panels.

[4] Gilles-Eric Séralini, Emilie Clair, Robin Mesnage, Steeve Gress, Nicolas Defarge, Manuela Malatesta, Didier Hennequin, Joël Spiroux de Vendômois (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

1. Introduction

The review presented in this statement is based solely on the details provided in the Séralini *et al.* (2012) publication since the complete study documentation is currently not available to EFSA. The Séralini *et al.* (2012) publication was reviewed taking into account good scientific practices such as internationally accepted reporting guidelines (Kilkenny 2010) and internationally agreed study guidelines (e.g. OECD guidelines for testing of chemicals[5]).

The Kilkenny *et al.* (2010) ARRIVE (Animals in Research: Reporting In Vivo Experiments) Guidelines for Reporting Animal Research detail how to report animal experiments covering the following areas: abstract, background, ethical statement, study design, experimental procedures, experimental animals, housing and husbandry, sample size, allocating animals to experimental groups, experimental outcomes, statistical methods, baseline data, number (of animals) analysed, outcome estimation, adverse events, interpretation/scientific implications, generalisability/translation and funding.

2. Overview of the study as reported in Séralini *et al.* (2012)

Séralini *et al.* (2012) report that the study followed 200 five-week old Virgin albino Sprague-Dawley rats over a period of two years. In total there were 100 female and 100 male rats used in this study. The rats were acclimatized for 20 days before they were randomly assigned on a weight basis into groups of 10 animals. Two rats of the same sex were housed together in a cage with a temperature of 22 ± 3°C and humidity of 45-65%. The rats had free access to feed and water, and litter was replaced twice weekly. Animals were monitored twice weekly with regard to general observation and palpation of animals, recording of clinical signs, occurrence of tumours, food and water consumption, and individual body weights.

Forty-seven biochemical parameters (from blood and urine) were measured on 11 occasions. The first measurement was taken before the administration of treatment (baseline) and the following measurements were taken at months 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24. Anatomopathology parameters were collected from 36 organs. Animals were sacrificed during the study due to suffering or for ethical reasons, otherwise pathology examination was performed at the end of the study. Histological examination was performed on nine organs (brain, colon, heart, kidneys, liver, lungs, ovaries, spleen, testes).

The treatments studied are three levels of glyphosate tolerant maize NK603 (GMO in the diet at 11%, 22% and 33%) treated and untreated with Roundup WeatherMAX® during its cultivation, its closest isogenic non-GM maize (Control in the diet at 33%) and Roundup® GT Plus (glyphosate based formulation referred as Roundup (R) in Séralini *et al.* (2012) at three increasing doses in drinking water. For each sex there were 10 treatment groups, each consisting of 10 rats, as follows:

1. Control 33% maize
2. GMO 11% maize
3. GMO 22% maize
4. GMO 33% maize
5. GMO 11% maize +R
6. GMO 22% maize +R
7. GMO 33% maize +R
8. R (A) (1.1 x 10^{-8}% of R)
9. R (B) (0.09% of R)

[5] Listed at http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

10. R (C) (0.5% of R)

Séralini *et al.* (2012) report pathological effects, in particular an increased tumour incidence linked to treatment with maize NK603 and R in both sexes.

3. Review of the Séralini *et al.* (2012) publication

In this section EFSA assesses the Séralini *et al.* (2012) publication, and highlights open issues that are usually addressed in a properly conducted, analysed and reported study.

3.1. Study objectives

Assessment

The study objectives are unclear in the Séralini *et al.* (2012) publication.

The objectives are the questions that the study is designed to answer. These questions must be pre-specified as the design of the study, sample size calculation, statistical analysis, study conduct and reporting are dependent on these. Depending on the objectives of the study different weight is given to the results in the context of a safety assessment. Without clearly stating the study objectives it is difficult to determine whether the study design and sample size used are fit for purpose or indeed what that purpose is.

International study guidelines are designed to meet specific objectives (e.g. OECD guidelines for chemical testing). If a specific guideline is chosen and followed then the objectives are inherently defined in the guideline.

Open Issues

- The study objectives need to be clearly stated *a priori* in the study protocol.

3.2. Study Design

Assessment

Séralini *et al.* (2012) did not follow the internationally accepted protocols for sub-chronic, chronic toxicity and carcinogenicity studies (e.g. OECD 408, OECD 451, OECD 452 and OECD 453) currently recommended in the EU for food and feed safety assessment. Given that Séralini *et al.* (2012) conducted a two-year study, it is unclear why an OECD guideline suitable for a two-year chronic toxicity or carcinogenicity study (i.e. OECD 451, OECD 452 or OECD 453) was not adhered to.

The strain of rats chosen is known to be prone to development of tumours over their life (Dinse (2010), Brix (2005), Kaspareit (1999)). By conducting the experiment on this strain of rats over two years, which is approximately their life expectancy, the observed frequency of tumours is influenced by the natural occurence of tumours typical of this strain, regardless of any treatment. This is neither taken into account nor discussed in the Séralini *et al.* (2012) publication.

The study design includes only one control group which is not suitable to serve as control for all the treatment groups. In particular, Séralini *et al.* (2012) claimed effects on the GMO 11%, GMO 11% +R, GMO 22% and GMO 22% +R without appropriate controls.

Séralini *et al.* (2012) draw conclusions on carcinogenicity by reporting on the incidence of tumours based on 10 rats per treatment per sex. There is a high probability that the Séralini *et al.* (2012) findings in relation to the tumour incidence are due to chance, given the low number of animals and the spontaneous occurrence of tumours in Sprague-Dawley rats. This is why relevant guidelines on

carcinogenicity testing (i.e. OECD 451 and OECD 453) recommend using at least 50 rats per treatment per sex. Given the limited number of animals and the chosen study design, no conclusions on the relationship between treatment and tumour incidence can be drawn from the Séralini *et al.* (2012) publication.

There is no mention of any measures taken to reduce the risk of bias such as blinding.

Open Issues

- The biological relevance of the rat strain used should be justified with respect to the design choices.
- Suitable controls for all treatment groups are not present.
- The sample size (power) calculation is not presented hence it is not possible to assess if the study was sufficiently powered to meet the unclear objectives.
- Measures taken to reduce the risk of bias (e.g. blinding) are not reported.

3.3. Feed and Treatment Formulation

Assessment

The publication states that "*all feed formulations consisted in balanced diets, chemically measured as substantially equivalent except for the transgene*". However, no detailed information on either the composition of the various diets used in the experiment or the storage conditions of the feeds over the course of the two years is provided. The publication does not give any details regarding the possible presence of harmful substances such as mycotoxins in the feeds used in the study.

Séralini *et al.* (2012) report only the application rate of the Roundup WeatherMAX® used to spray the plants and the concentration of the Roundup® GT Plus added to the rats' drinking water. They state that the consumption was measured though it is not reported. Without this information it is not possible to estimate the exposure level. Furthermore, the level of residues of glyphosate and its metabolites on treated maize are not specified. Hence, their contribution to the reported findings cannot be assessed. In addition, information on other chemical contaminants e.g. other pesticides applied on the GM maize as well as on the isogenic non-GM control maize, is not provided.

Open Issues

- The appropriateness and comparability of the diets cannot be assessed as critical information about their composition is not reported.
- The stability of the diets cannot be assessed as details of their storage conditions are not provided.
- It is impossible to evaluate whether or not there was any contamination of the diets, e.g. by mycotoxins, as it is not reported.
- The amount of residues of glyphosate and its metabolites in treated maize NK603 is not reported.
- The exposure to GMO, GMO +R and R cannot be evaluated since the food and water intakes of the GM- and R-treated groups, respectively, are not clearly reported.

- Suitability of the control cannot be determined because information on the possible exposure to other chemicals.

3.4. Statistical Methods

Assessment

It is not reported if the statistical analyses were pre-specified in the protocol (i.e. prior to the start of the study) or in a statistical analysis plan prior to any access to the data.

Summary statistics for all measured parameters (including biochemical and tumour related) by treatment group and sex are not presented.

Séralini *et al*. (2012) have chosen an unconventional statistical methodology to analyse the biochemical parameters instead of commonly used methods (e.g. analysis of variance). The methodology chosen does not allow for the estimation of the (unbiased) treatment effects and their associated variations.

Séralini *et al.* (2012) only present percentages and graphical summaries of the tumour incidences. There is no modelling-based analysis (e.g. time to event analysis) to estimate the (unbiased) treatment effects and their associated variations. For both types of analysis the issue of missing data and multiplicity should also be addressed.

Open Issues

- It is not clear if the analysis presented is consistent with any pre-planned analyses.
- The reported analysis does not provide the following information needed to draw conclusions:
    - A summary of drop outs and censoring (e.g. euthanised animals).
    - Summary statistics for all measured parameters by treatment group (and sex).
    - Unbiased treatment effect estimates (with confidence intervals) derived from an appropriate statistical analyses for the chosen design and endpoint. The issues of handling missing data and multiple testing (multiplicity) should be addressed.

3.5. Endpoint Reporting

Assessment

Far more endpoints (and measurement points thereof) than those reported in the publication were collected by Séralini *et al.* (2012). It is unclear why the publication does not report the complete set of samples collected and endpoints measured.

Clinical observations other than tumours are selectively reported: in Table 2 of Séralini *et al.* (2012), a summary of the most frequent anatomical pathologies observed is presented; however a clear presentation of all the specific lesions occurring in the different organs, for each treatment group, is not provided.

As for the carcinogenicity assessment, attention was mainly focused on the "largest palpable growths" with only mammary and pituitary tumours being mentioned for females and kidney and skin tumours for males. A detailed list of all tumour types per sex per group and notation of all histopathological lesions (including hyperplastic, pre-neoplastic and non-neoplastic) would be needed.

Open Issues

- All collected endpoints should be reported openly and transparently.

CONCLUSIONS

EFSA notes that the study, as described in the Séralini *et al.* (2012) publication, is inadequately reported with many key details of the design, conduct, analysis and reporting being omitted. Without such details it is impossible to give weight to the subsequent results.

Conclusions cannot be drawn on the difference in tumour incidence between the treatment groups on the basis of the design, the analysis and the results as reported in the Séralini *et al.* (2012) publication. In particular, Séralini *et al.* (2012) draw conclusions on the incidence of tumours based on 10 rats per treatment per sex. This falls considerably short of the 50 rats per treatment per sex as recommended in the relevant international guidelines on carcinogenicity testing (i.e. OECD 451 and OECD 453). Given the spontaneous occurrence of tumours in Sprague-Dawley rats, the low number of rats reported in the Séralini *et al*. (2012) publication is insufficient to distinguish between specific treatment effects and chance occurrences of tumours in rats.

Considering that the study as reported in the Séralini *et al.* (2012) publication has unclear study objectives and given its inadequate design, analysis and reporting, EFSA finds that it is of insufficient scientific quality for safety assessments. Therefore EFSA, concludes that the Séralini *et al.* study as reported in the 2012 publication does not impact the ongoing re-evaluation of glyphosate, and does not see a need to reopen the existing safety evaluation of maize NK603 and its related stacks.

NEXT STEPS

To review the study in more detail, beyond what is reported in the Séralini *et al.* (2012) publication, access would need to be given to the study documentation and procedures followed, including the original study protocol, along with documentation on any planned or unplanned changes to it, the statistical analysis plan, the statistical report/analyses and the final full study report. Therefore, the authors will be made aware of the content of this EFSA statement and will be given the opportunity to submit information to EFSA.

A second EFSA output will cover all the ToR and will take into account any information received from the authors, the already ongoing assessment activities from the Member States (such as Belgium, France, Germany[6] and The Netherlands[7]) and the assessment of the German authorities responsible for the evaluation of glyphosate.

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