Food and Chemical Toxicology xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Letter to the editor

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Response to original research article, in press, corrected proof, "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize" Available online 19 September 2012, Gilles-Eric Séralini, Emilie Clair, Robin Mesnage, Steeve Gress, Nicolas Defarge, Manuela Malatesta, Didier Hennequin, Joël Spiroux de Vendômois.

We have reviewed the aforementioned article and have found numerous deficiencies in the way the study was designed, and in the manner in which the data were presented and analyzed. As a consequence of these deficiencies, the study cannot be used to support any conclusions regarding the safety of NK603 glyphosate tolerant maize and Roundup® herbicide.[1]

1. Experimental design

The authors of this study assert that it was conducted in a GLP environment and according to OECD guidelines. They did not follow OECD GLP guidelines nor OECD testing guideline (TG) 453 for conduct of a combined chronic toxicity/carcinogenicity study. OECD GLP's require "Detailed information on the experimental design, including a description of the chronological procedure [e.g., start date, end date] of the study, all methods, materials and conditions, type and frequency of analysis, measurements, observations and examinations to be performed, and statistical methods to be used (if any)" and . . . "The study should be conducted in accordance with the study plan". Apparently, the authors' original intent was not to conduct a carcinogenicity study "...we had no reason to settle at first for a carcinogenicity protocol using 50 rats per group." (Seralini et al., 2012), but at some point during the in-life phase, they changed the purpose of the study by extending it for 2 years to assess potential carcinogenicity. Assuming they had a protocol at the start of the study, they did not follow it as they substantially altered the purpose and the design of the study while it was in progress. This should be considered a violation of GLP guidelines as the study was not conducted in accordance with the original study plan. If they wanted to carry out a carcinogenicity study, they should have terminated the existing study, and prepared a new study plan adapted from OECD TG 453. They did recognize, as stated above, that they needed a larger number of animals (a minimum of 50 rats/sex/group) for a carcinogenicity study, instead of the 10 rats/sex/group that they had in their existing study. For reasons which will be discussed later, their study did not have enough animals to draw any meaningful conclusions.

Rodent carcinogenicity studies must be sufficiently powered not only to detect an increased incidence of rare tumor types, but also to discriminate treatment-related effects from spontaneous, or background, incidence of common tumor types. For this reason, US (US EPA, 1998; FDA, 2006) and OECD (1995a) regulatory guidelines for the conduct of carcinogenicity studies in rodents specify the use of at least 50 animals per sex per treatment group. In addition, OECD states that "it is unlikely that a regulatory authority would find a study using a lower core number of animals per sex and per group acceptable for regulatory purposes, since a sufficient number of animals should be used so that a thorough biological and statistical evaluation can be carried out." (OECD, 1995b). OECD further states that "for strains with poor survival such as SD rats, higher numbers of animals per group may be needed in order to maximize the duration of treatment (typically at least 65/sex/group)."(OECD, 1995b). For this reason, the US EPA specifies that survival in any group should not fall below 50% at 18 months or below 25% at 24 months (US EPA, 1998), while the US FDA specifies survival of a minimum of 25 rats per sex per group at study termination (FDA, 2006). The SD rat has been widely used in toxicology research, including numerous chronic studies, but these studies employ many more animals than used by the authors in consideration of their lower survival rate and high background tumor rates, especially mammary tumors in females.

2. Statistical analysis and presentation of data

The authors have a history of inappropriate application of statistical methods to analyze toxicology data (Séralini et al., 2007; Spiroux de Vendômois et al., 2009) which has been criticized by regulatory agencies and other experts (EFSA, 2007, 2010; FSANZ, 2009a,b; HCB, 2009; Doull et al., 2007). There are numerous problems in the way the data were statistically analyzed in this study.

For example, in Table 3, mean values are not presented for each group and sex to allow comparison of measured parameters. Control data are not presented. Instead, the authors used a statistical method that is not traditionally used to present toxicology data, a multivariate technique called Partial Least Squares Discriminant Analysis (PLS-DA). Mean differences (%) of variables (discriminant at 99% confidence intervals) were presented to investigate the relationship among 48 blood and urine measurements relative to the different treatment groups. PLS-DA can be used to identify patterns in the data and to develop a function which can be used to discriminate between the groups. However, any differences between groups must be further evaluated for toxicological relevance. Presentation of the data in this manner does not lend itself to straightforward interpretation of the study findings.

In Fig. 5, the same PLS-DA procedures were followed with jackknifed confidence intervals at 99% confidence level. This procedure may be familiar to statisticians, but it is not commonly used to

☆ DOI of original article: http://dx.doi.org/10.1016/j.fct.2012.08.005

[1] Roundup agricultural herbicides are registered trademarks of Monsanto Technology, LLC.

http://dx.doi.org/10.1016/j.fct.2012.10.044

present toxicology data and is difficult to interpret, particularly when the data used to construct these graphs are not presented. Examination of Fig. 5a would suggest that the majority of measured parameters fall within 99% confidence intervals with the exception of serum and urine electrolytes. Unfortunately, no data were provided from other intervals when these data were collected to determine if the same patterns were evident. No lab historical data were provided to put these data in perspective. As stated earlier, just because one can discriminate between the groups, it does not make the result toxicologically relevant. There was no presentation of actual statistical analysis to compare the means for each measured parameter.

To determine if there are patterns of differences in toxicologically related findings, the toxicologist expects to see the actual mean data for each parameter/group and the standard deviation and the control data should also be provided for comparison. The test and control values for measured parameters should also be compared to the historical control data from the testing laboratory and/or the literature to determine if differences were within or outside of the normal range. As presented, the reader has no way of determining whether the conclusions drawn by the authors are supported by the actual data, or are merely statistical anomalies resulting from non traditional analysis. The manuscript contained figures with graphs that were difficult to read because lines overlapped, and percent variations were presented rather than the mean test and control data which is the more standard practice in presenting toxicology data. For instance, incidences of 1 vs. 2 or 5 vs. 10 both represent a change of 100%, however, these absolute values would likely result in different conclusions.

The same criticism can be made for Fig. 2 and Table 2 where the data are not broken out in the tables so the reader can actually see what changes were observed for each group. The incomplete presentation of study data, which was acknowledged by the authors – "all data cannot be shown in one report, and the most relevant are described here-" precludes meaningful review and evaluation of study results (Seralini et al., 2012). For example, histopathology incidence/severity data are not presented (e.g., Table 2); nor is any laboratory historical control data provided to help interpret the biological relevance of clinical pathology and histopathology findings. Did the testing laboratory have historical pathology data for chronic studies? The generalized statements of increased liver disorders cannot be verified without presenting the actual data in a table to review.

3. Misinterpretation of study findings

3.1. Mortality data

The authors stated that male and female rats in all treatment groups had more and earlier deaths than the controls. However, they acknowledge that mortality was not dose related. For example, according to Fig. 1, low dose males fed NK603 grain (unsprayed with Roundup) had more early deaths and overall mortality (5/10), while the mid and high dose group mortality near the end of the study was similar to controls (3/10). In the male group fed NK603 (sprayed with Roundup), the mid dose males had more early deaths (4/10), followed by the low dose, and the high dose had the lowest mortality of the NK603 fed groups. For rats administered Roundup in drinking water, high dose males had the lowest mortality compared to the other Roundup treated groups. Similar examples of lack of dose relationships in mortality were observed in the treated female groups. In consideration of the fact that there were 9 treatment groups compared to one control group, some variability in mortality between groups would be expected by chance and could well have explained the distribution of mortality in the study. Given the small group size of 10 rats/sex/group, differences in mortality between groups generally involved only a few animals, and it would be difficult to interpret the biological relevance of such small differences. If dose is not important in this design, it is a 90% probability that one of the test groups would numerically have the highest incidence of mortality.

The authors should have used the adjusted analysis of survival to determine if there were more dead animals in the treated groups compared to the control group, and if there were earlier deaths in the treated groups than in the control group. The most useful statistical approach used to compare survival between groups (not followed by the authors) is the following procedure: Adjusted survival rates are estimated using Kaplan Meier estimation procedures (Kaplan and Meier, 1958). Kaplan Meier estimates are calculated separately for each sex and treatment group. Mortalities which are the result of animals dying following accidents (accidental trauma, died during anesthesia, killed at study director request) or at scheduled sacrifice have to be considered as censored observations. In a second step, statistical significance of differences in survival rates between treated and control groups and dose related trend in survival could be assessed using Cox's and Tarone's tests on life table data.

The authors did not indicate whether the tumor classification was done according to the PETO codes (incidental, fatal, observed in life). At least a PETO analysis or a mortality-adjusted analysis for tumor incidences should have been performed.

The authors reported higher survival than is typically reported for female Harlan SD rats in 2-year studies. According to Fig. 1, only 2 of 10 animals died before the end of the study resulting in survival rate of 80%. The SD rat is known to exhibit low and variable survival after 18 months of age (Nohynek et al., 1993; Keenan, 1996). Therefore, as discussed earlier, many more animals than 10/sex/group would be needed to ensure that there would be a sufficient number surviving to the end of the study. This would be needed to conduct a meaningful statistical analysis and to draw solid conclusions regarding biological significance. Average survival in 7 NTP 2-year studies with female Harlan SD rats was reported to be 41.5% (Brix et al., 2005). In a later published review, a survival rate of 42.5% was reported for 2-year studies conducted by the NTP with female Harlan SD rats (Dinise et al., 2010). Charles River SD female rats were reported to have a 2-year survival ranging for 20–60% with an average of 37% (Giknis and Clifford, 2004). Given the high survival rate of female rats in this study, it would be very interesting to learn what the historical 2-year survival rate was for female Harlan SD rats in the testing facility that performed the authors' study. No historical control data from the testing laboratory were provided for any of the parameters measured.

3.2. Tumor findings

The manuscript misleads readers by attributing the tumors observed in the study to treatment with NK603 grain administered in the diet or Roundup via drinking water. For example, the authors failed to acknowledge that mammary and pituitary tumors observed in this study are very common in untreated female SD rats fed *ad libitum* for 2 years. They included color pictures of treated rats bearing large mammary tumors, but did not did not include photos of control rats or acknowledge that similar tumors were also observed in controls. Mammary gland tumors are observed not only in older control female SD rats, but can also appear early in a chronic study (Durbin et al., 1966). Older control female Harlan SD rats have a high background tumor incidence, e.g., for the mammary gland, adenoma 3%; adenocarcinoma 11%; fibroadenoma 71%; adenomas of the pituitary gland are reported at an incidence of approximately 41% (Brix et al., 2005). Pituitary adenomas (prolactinomas) contribute to the development of mammary tumors in SD rats. These historical observations can ac-

count for the finding of one mid dose female in the mid dose NK603 group (unsprayed) exhibiting a mammary tumor earlier in the study, and the other mammary and pituitary tumors observed in both control and treated female groups later in the study. In Table 2, the authors report that treated females had more mammary tumors/rat than controls. However, they do not follow the standard convention of listing the tumor types confirmed pathologically for each group and incidence of animals in each group bearing those tumors. The authors have instead combined all of the tumors together/animals in a group so the reviewer cannot compare the actual tumor data by type between groups. The absence of a dose relationship in some of tumor findings was evidenced by the high dose Roundup group females having lower incidence of total tumors than the low dose group. The authors also noted that the size and number of tumors were not proportional to the treatment dose. Since the low dose of the high dose, yet the lowest dose had a higher tumor incidence, the Roundup administered in drinking water was orders of magnitude lower than data are clearly not dose related and most likely reflect normal variability in the incidence of common tumors that have a high background rate.

3.3. Other pathologic findings

Other pathological changes reported by the authors as treatment-related are similarly prevalent in the aged SD rat, including multiple diet-related disorders, degenerative renal and endocrine diseases, etc. (Keenan, 1996).

The authors reported treatment-related liver and kidney pathologies in males. As evidence of kidney effects, they refer to Table 2 where the incidence of chronic progressive nephropathy (CPN) was 3/10 control animals compared to 7/10 animals in the high dose NK603 group (non-sprayed). However, they neglect to mention that the incidence of CPN in the NK603 sprayed groups and the Roundup groups are similar and that the high dose groups had the lowest incidence. They did not report the severity grades of CPN to learn whether it was increased in a dose related manner. A similar pattern was observed for liver findings, although Table 2 does not state what the liver pathologies were. This is an unacceptable way to present pathology data. As the study progressed, there were insufficient numbers of male animals left to make meaningful comparisons for liver and kidney pathology changes. The authors reported that only 3/10 control male animals were found to have CPN. This pathologic change has been reported to occur commonly in male rats (Hard and Khan, 2004) and in one chronic rat study with Harlan SD male rats, the incidence was 100% in control male rats (Petersen et al., 1996). One might have expected a higher incidence of CPN in control males. In Petersen et al. (1996), CPN accounted for 48% of the early deaths in control males. Given the very high background incidence of this disease, and the fact that 9 treatment groups are being compared to one control, some variation in the number of CPN afflicted animals would be expected between groups. Unfortunately, no historical control lab data for pathologic lesions were made available for comparisons. The author's misquoted the aforementioned Hard and Khan (2004) publication stating that only elderly rats are sensitive to CPN whereas the publication states "Although usually regarded as a disease of the aging rat, incipient lesions of CPN are detectable in hematoxylin and eosin (H&E)-stained sections of male rat kidney at least as early as 2 months of age."

The authors have asserted in previous publications (Séralini et al., 2007; Spiroux de Vendômois et al., 2009) that GM crops cause liver and kidney pathologies based on their statistical reanalysis of published 90 day feeding studies mentioned earlier. However regulatory agency scientists and other experts have not supported these claims and find no evidence of treatment related liver or kidney pathology changes in any of these studies (EFSA, 2007, 2010; FSANZ, 2009a,b; HCB, 2009; Doull et al., 2007).

The authors also presented clinical pathology data in Fig. 5 and Table 3 which they interpreted to show changes in serum and urine electrolytes supporting their hypothesis of kidney damage. However, as stated earlier, the presentation of the data does not permit comparison of the actual measured values to controls since control data were not presented. No actual mean data for the urine and serum electrolytes were provided to provide comparisons between test and control groups as well as historical control ranges for these parameters from the testing laboratory.

3.4. Glyphosate safety

Since a number of the changes observed in this study were not dose related, the authors conjectured that these findings were hormone and sex dependent, and exhibited a threshold response at a single dose, which happened to be the lowest dose tested. They state categorically that Roundup is a "sex endocrine disruptor" that contributed to the tumors and other pathologies observed in their study, with no scientific basis for this statement.

To respond to these allegations, it is necessary to review what is known about the potential toxicology of Roundup and its active ingredient, glyphosate. WEATHER MAX® herbicide is a typical commercial Roundup formulation that is essentially the potassium salt of glyphosate with 10% surfactant in water. The category of surfactant in this Roundup™ formulation was evaluated by the US EPA in 2009 and was considered acceptable for this use in pesticide products based on the results of multiple repeat dose studies, including reproductive and developmental toxicology (US EPA, Federal Register, 2009a). It should further be noted that consumers have regular exposure to surfactant materials in the form of shampoos, soaps, and cleaning products. These are similarly not believed to present reproductive/endocrine risks, but in any event, exposure to surfactant residues as a result of pesticide exposure represents a very small portion of human surfactant exposure. There is no evidence that the surfactant categories used in Roundup are endocrine disruptors (Williams et al., 2012).

Glyphosate is a structural analog of the amino acid glycine, it has a methylphosphonate group at the amino terminus instead of a carboxyl group. Amino acids are not endocrine disruptors. Extensive *in vitro* (test-tube) and animal data indicate glyphosate is not an endocrine disrupter. Although glyphosate was included in the EPA's initial substances for the endocrine disrupter screening program, EPA has stated "This list should not be construed as a list of known or likely endocrine disruptors. Nothing in the approach for generating the initial list provides a basis to infer that by simply being on this list these chemicals are suspected to interfere with the endocrine systems of humans or other species, and it would be inappropriate to do so." (US EPA, Federal Register, 2009b). Furthermore, the EPA specifically rejected the assertions presented in Richard et al. (2005) that glyphosate was an endocrine disruptor based on (i) exceedingly high doses, over 40 times the maximum acceptable concentration for this study type, (ii) failure to actually meet the criteria for a positive result in this assay, despite the high dosing, and (iii) lack of demonstrated study proficiency including no concurrent positive controls to demonstrate assay validity (US EPA, 2011).

The cited *in vitro* studies conducted by the Seralini laboratory have repeatedly been reviewed and considered irrelevant to *in vivo* exposures by numerous authoritative bodies. *In vitro* test systems are not appropriate for evaluating surfactants due to their physico-chemical properties impairing cell membrane integrity, including mitochondrial membranes. The selective use of literature, without consideration of research (Levine et al., 2007) demonstrating that the effect is the result of surfactant impacts

on mitochondrial membranes and occurs with a range of surfactants, including those with much greater consumer exposure, demonstrates consistent and undeterred bias in the authors' publication record. Numerous authoritative body reviews have discounted the relevance of the Seralini team's research to human health risk assessment; such as, French Ministry of Agriculture and Fish, Committee for Study of Toxicity (2005), French Agency for Food Safety, AFSSA (2009), and BfR (2009).

The safety of glyphosate has been assessed in numerous chronic/carcinogenicity studies conducted by various registrants over the years, as glyphosate has gone off-patent, and none of these studies have found any evidence that glyphosate causes mammary cancer or any other kind of cancer. The WHO/FAO Joint meeting on Pesticide Residues reviewed several glyphosate toxicology data sets including five chronic rat and two chronic mouse studies in 2004, concluding no evidence of carcinogenicity (WHO/FAO, 2004a,b). The US EPA's classification as "Group E carcinogen (signifies evidence of non-carcinogenicity in humans)" is based on review of two chronic rat and one chronic mouse study (US EPA, 1993) and the EU Commission conclusion of "no evidence of carcinogenicity" is based on review of four chronic rat and four chronic mouse studies (EC, 2002). The dosages used covered a broad range of exposures, and the highest dosages used were much greater than those tested by the authors and many, many times higher than human potential exposures since glyphosate can be dosed at high levels in animals as it is not very toxic. Thus, the overwhelming weight of evidence indicates glyphosate is not an animal carcinogen.

In the authors' chronic study, there were 20 control and 180 test rats (sexes combined) divided into 9 different groups. In contrast, the FAO/WHO (2004b) review of glyphosate referenced above included a total of 2330 rats in 5 chronic rat studies. Included in this number were 540 control rats. In the recent EU Annex 1 Renewal dossier submitted in Europe for glyphosate, there were 9 chronic rat studies with a total of 3938 rats (additional studies from new manufacturers of glyphosate) of which 942 were control rats. The new chronic studies also reported no evidence of carcinogenicity. The authors failed to mention the many toxicology studies carried out on glyphosate that confirm it does not cause cancer or liver and kidney pathologies as reported by the authors.

The authors did not acknowledge that there was another chronic rat study carried out with glyphosate tolerant soybeans where the investigators reported no evidence of treatment-related adverse effects including cancer. This was a more robust study as it contained 50 rats/sex/group (Sakamoto et al., 2008).

The authors also reported blood hormonal analyses (estradiol, testosterone), although no specified times during the day were given for blood sampling. Hormonal parameters exhibit significant diurnal variations. For this reason, proper analysis must include the historical variation observed in the performing laboratory, but no information was provided in this study – a very significant omission. Secondly, the results of hormone analysis on just one day are not representative of what is going on throughout the study, especially for hormones characterized by episodic secretion. No dose–response relationship in hormone levels was observed. It is not possible to correlate the hormone levels observed at one time point in this study with the development of mammary tumors as proposed by the authors. Further, in rats, the main mode of action for development of mammary tumors is an increase of prolactin level and then an increase of pituitary tumors. Thus, we question the increase of tumor incidence with concomitant decrease of estradiol and increase of testosterone. It is not logical.

The authors also propose another hypothesis to explain their data, that the introduction of the CP4 EPSPS enzyme that imparts tolerance to topically applied glyphosate caused metabolic disturbances in secondary metabolites. In particular, they report a statistically significant reduction in the levels of secondary metabolites caffeic and ferulic acid in the NK603 diets. The levels of ferulic acid in the NK603 diet (exact diets not specified) were reported to be from 735 to 889 ppm compared to 1057 ppm in the control. Since they report differences in the diets, it is unclear whether other ingredients in the diet could have contributed to these differences. No details were provided on the dietary components in the formulated diets except the level of NK603 and control grain that were added.

In a published study summarizing compositional analysis of NK603 grain, Ridley et al. (2004) reported no differences in ferulic acid levels between NK603 and its control comparator. The range of grain ferulic acid was 1500–2500 ppm (mean 2000 ppm) for glyphosate sprayed NK603 maize. Control maize levels ranged from 1700 to 2300 ppm (mean 2000 ppm). Ferulic acid levels can vary considerably in non GM maize ranging from 174 to 3540 ppm (fw) with a mean of 1950 ppm (ILSI Crop Composition Data Base, v4.2).

3.5. Questions on EM methods

The authors reported finding glycogen dispersion or appearance of lakes, etc. following electron microscopic (EM) examination of livers from animals fed NK603 (sprayed) or animals administered Roundup in drinking water. Manuela Malatesta, who performed the EM work described in this publication, has been previously criticized for technical deficiencies regarding EM work carried out in mice fed presumably glyphosate tolerant soybeans (Williams and DeSesso, 2010).

The authors do not describe the fed/fast state of the animals at the time of terminal killing. The liver is a dynamic organ that stores and releases glycogen quickly. Different feeding states of animals in the same treatment/control group could give samples that look like all three micrographs in Fig. 4.

The authors' statements regarding the quality of the methods used are not backed up by the description in the publication. The electron microscopy is based on an unknown number of samples from one control, one low dose and one mid dose animal. These animals were reported to exhibit the greatest degree of liver pathology yet the authors report no procedures to ensure a balanced investigation of treated versus control samples. The micrograph of the control portion of a hepatocyte shows tissue from an area $13 \times 13\ \mu$. The total area is of the picture is the area is about the size of 3 red blood cells. This is a very small amount of tissue on which to draw a conclusion.

The most significant issues with the limited amount of selective microscopy used to support the authors' contentions relate to the anatomy of the liver. The liver is a large organ (the largest internal organ in the body) that has great diversity in its anatomy. If a sample were taken from the edge of the liver and were compared to a sample from the middle of the same liver near the entry of the portal vein, the cells would look different. The fact that the tissue was diced and not put in fixative precludes knowing whether the samples were taken from the same section of organ across all treatment groups.

Not only is the liver diverse across the organ, but also within its internal structure. One of the ways histologists describe the organization of the liver is by speaking about the liver lobule. For the purpose of this discussion, the method that describes a liver lobule as liver cells surrounding the central vein of the lobule will be used. In that description, the lobule is conceptualized as consisting of three concentric layers of cells that surround the central vein in a hexagonal shape. (There are thousands of these lobules in a lobe of the liver.) The arterial supply to the liver lobules is derived from arteries at the angles of the hexagon. In the fed state, glucose arrives via the arteries and is processed into glycogen by the hepatocytes. The outer layer takes up glycogen first; later the middle

layer will take up glycogen; and finally, if sufficient glucose is left, glycogen will be found in the inner layer. Glycogen stores are depleted in reverse order. Consequently, the innermost layer tends to look glycogen-depleted most of the time; under fed conditions the outer layer has many glycogen granules; and the middle layer is intermediate in appearance. One could find all three of the conditions illustrated in Fig. 4 by looking within a single (or several) lobules from the same tissue sample. Mitochondria also have various appearances depending on their proximity to the oxygen rich arteries or oxygen depleted central vein.

In the absence of rigorous morphometric analysis that also accounts for the anatomy of liver lobules, the photographs in Fig. 4 have neither context nor toxicological meaning,

In Fig. 3, necrotic foci are considered to be either clear focus or basophilic focus: which is scientifically wrong as these foci are preneoplastic entities. Moreover basophilic focus with atypia is not part of the international microscopic nomenclature. Furthermore, microscopic pictures cannot be interpreted properly (bad quality and low magnification). Macroscopic pale spots cannot be correlated to a necrotic focus.

3.6. Questions regarding materials and methods, missing data

No information was provided regarding the identification of the near isoline to confirm that it had similar genetic background. The location, growing conditions, watering and agrochemical treatments of crops were not detailed. This could have had an impact on the composition of crops and then on the outcome of the study.

No information was provided on the potential mycotoxins that might be found in the control and NK603 treated crops and might have impacted the study. Was the grain stored adequately during the 2 years of the study to minimize mold growth and mycotoxin contamination? How often were batches made, were they checked periodically by PCR methods to confirm that the control diets contained only control and not test maize and visa versa. How were the diets stored?

No information was provided regarding (a) detailed diet formulation and manufacturing processes as well as nutrient composition of the diets (b) drinking water contaminant analysis methods or results (c) homogeneity, stability or concentration of ROUNDUP in drinking water formulations. How often were drinking water solutions produced?

The control group was reported to contain 33% non-GM maize in the diet. Low and mid dose NK603 groups (sprayed, unsprayed) reportedly contained 11% and 22% NK603 maize grain. Results from the low and mid dose groups cannot be compared to the control group if they had lower levels of corn grain added to the diets.

There was no drinking water control group for comparison to the treatment groups fed different concentrations of Roundup in drinking water.

3.7. Missing data

In Table 1, the study design represents that behavioral studies were conducted twice. There is no mention of behavioral studies in methods and no results were presented.

Ophthalmology was reported to be conducted twice. There is no mention of ophthalmology evaluations in the methods and no results were presented.

Microbiology was to be conducted in feces and urine. There is no mention of microbiology evaluations in the methods and no results were presented.

Evaluation of glyphosate residues in tissues was reported to be performed, but no information on methods or data generated was provided. Tissue residues are usually evaluated after administration of radiolabelled test materials under toxicokinetic testing guidelines such as OECD 417 (OECD, 2010). For glyphosate, the results of such studies have been evaluated by the WHO/FAO Joint Meeting on Pesticide Residues (2004a,b) and other regulatory agencies around the world.

Evaluation of the transgene in tissues was reported. There was no mention of transgene analysis in methods or results sections, with the exception of confirmation NK603 in maize grain and formulated diets by qPCR.

Food, water consumption and body weights were reported to be measured in the study, but the data were not presented in the manuscript. This is basic information that should be provided for a chronic feeding study to assess potential adverse effects.

Clinical pathology data was reported to be measured at eleven different intervals during the study but only data from month 15 was summarized, and not in a manner it could be easily reviewed. Further, data from the two sexes was presented differently. No historical control information from the testing laboratory for measured parameters was presented.

4. Conclusion

As a result of methodological failures, incomplete data presentation, and lack of proper statistical analysis, Seralini et al.'s conclusions regarding NK603 and/or Roundup cannot be supported by the presented data. Indeed, the fundamental flaw in regards to the number of animals employed makes it highly unlikely that any of the purported findings can be statistically supported using standard approaches to analysis even if more data were to be provided by the authors.

Conflict of Interest

The authors declare that there are no conflicts of interest.

5. Uncited references

(EFSA (2008); EFSA (2011); Ministere De L'Agriculture et de la Peche (2007); Hammond et al. (2004); ILSI (20110; OECD (2009); WHO/FAO (2004); Williams et al. (2012)). Q3

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