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From: Brian John [brianjohn4@mac.com]
Sent: 07 October 2012 23:28
To: LEFFLER-ROTH Carolyn
Cc: EVANS Jill OFFICE; LEPAGE Corinne
Subject: Petitions 0436/2010 and 0813/2008 and EFSA conspiracy

Attachments: Seralini_et_al_final_paper.pdf; EFSA review of Seralini paper.pdf; Neths Ministry.pdf; ATT5479686.txt


Seralini_et_al_final_paper.pdf...


EFSA review of Seralini paper....


Neths Ministry.pdf (4 MB)


ATT5479686.txt (5 KB)

Carolyn Leffler-Roth

Petitions Committee
European Parliament

7th October 2012

Dear Carolyn

Petitions 0436/2010 and 0813/2008 EFSA

EFSA conspiracy to "bury" Seralini rat feeding study

With reference to my two Petitions, you will recall that I wrote to you on 10th May asking that the two Petitions be kept alive, on the basis that EFSA had not adequately addressed my concerns, or those of my colleagues who joined me in Petition 0436/2010. In our view EFSA still acts in a manner that places the facilitation of GM consents above the protection of public health -- and in that regard it is still unfit for purpose. In my view, and that of many NGOs, the EFSA GMO Panel must be dissolved and reconstituted with a fresh group of individuals who have due regard for scientific ethics and the Precautionary Principle.

I now ask you to bring this matter back to the Petitions Committee as a matter of urgency, following the quite extraordinary manner in which EFSA has dealt with the new paper by Seralini and his team regarding the health effects of NK603 maize and Roundup on experimental mammals. (See PDF number 1, below) The French team showed that both the GM maize and Roundup residues (at low concentrations in drinking water) caused chronic toxic effects in experimental rats, including a greatly increased incidence of tumours. As many of us had predicted, as soon as EFSA got wind of the Seralini group's study, it slipped into "damage limitation" mode, apparently determined from the outset to find fault with the paper and to discredit the authors. This was in spite of the fact that the paper was published in a reputable peer-reviewed journal, and in spite of the fact that there were no "red warning lights" during the peer review process. In other words, the research findings were more honestly reported, analysed and scrutinized than any of the non-replicable and non-verifiable studies used in the approvals process for either NK603 or for Roundup herbicide. The paper should have been given careful consideration, just as Prof Seralini and his colleagues should have been accorded due respect as reputable and experienced scientists.

(After all, Prof Seralini has been a member of two French government commissions on GMOs (the Biomolecular Engineering Commission which oversees risk assessment, and on which he served for nine years, and the Biovigilance Committee looking at commercialised GMOs, on which he served for ten years). In 2003 he was appointed an expert advisor on GM to the European Commission in the context of its WTO dispute. And in 2008, Prof. Seralini was made a Knight of the French Order of Merit in recognition of his scientific research. He is neither a maverick nor a "campaigning environmentalist" -- and he knows far more about GM toxicity and animal feeding trials than any of the EFSA secretariat and GMO Panel members who are now involved in a campaign of personal vilification against him.)

I have looked at the EFSA review of the publication by Seralini et al, and find it to be full of points that are frankly quite stupid. They seem to have been copied and pasted from other "expert" opinions fed to them by the Science Media Centre and other

organizations. (See PDF number 2, below) The whole review is disrespectful, complacent and disingenuous. This is not surprising, since it was written, under the guidance of Per Bergman, by a small group of employees comprising Saghir Bashir, Danièle Court Marques, Claudia Paoletti, Manuela Tiramani, Didier Verloo and Elisabeth Waigmann, with reviews by Andrew Chesson (a member of the GMO Panel) and Alberto Mantovani (a member of the PPR panel). Quite incredibly, Andrew Chesson was one of those who made the original assessment of NK603 for EFSA in 2003! This was presumably the "multidisciplinary task force" charged with the task of damage limitation and asked to attack the Seralini study on as many fronts as possible. There was no way that EFSA could have contemplated anything different, since to admit to any merit in the Seralini study would have been to admit to serious shortcomings in the initial EFSA assessment of NK603 and to major failings in the EU assessment of Roundup herbicide as well. EFSA therefore decided to be judge, jury and executioner, and to align itself with the GM industry spokesmen in seeking to bury the Seralini study and to discredit its authors.

I now accuse EFSA of conspiring on 28th September 2012 with a small group of selected civil servants from four member states (France, Belgium, Netherlands, and Germany) to come to a common view on the Seralini study and to eliminate or suppress dissenting views (1). Where did their mandate come from, and by what right do they purport to give an authoritative EU assessment of the paper in question? Have the other member states been consulted, and have they agreed that their scientific opinions should be represented by the named individuals from these four states which have a long record of supporting GMOs? In the enclosed PDF number 3 (below) you will see, in the English-language minutes starting on p 21, that this group of carefully-selected and unelected civil servants from various ministries presumes to speak not just on behalf of EFSA but for the EC as well, since the EC asked for the EFSA opinion in the first place. As far as I can see, the "EFSA opinion" determined by this small group was promoted via an EFSA press release on 4th October without any approval from the member states, in the full knowledge that it would be accepted by a gullible media as the authoritative statement of the EFSA position. This is an outrageous abuse of power by this small group of civil servants who know next to nothing about the practicalities of long-term safety studies involving the use of mammals.

The strategy by Per Bergman to "avoid divergence" shows a complete disregard for scientific ethics and also a failure to understand how science works. It tells us a great deal about the prevailing mindset within EFSA.

I repeat -- this is a deliberate attempt to cover up the shortcomings of the EFSA assessment process for NK603 (and other materials which have obtained EC consents), to "rubbish" the recent rat feeding study, and to destroy the credibility of Professor Seralini and his team. This conspiracy is serious enough, but it pales into insignificance when set against the key findings of the Seralini study -- namely that NK603 maize and Roundup are toxic to mammals. These findings are not aberrations -- they simply confirm the evidence that has been coming into the public domain over the last decade from many different sources -- and especially from independent studies conducted in the face of unremitting hostility from the GM industry and from bodies like EFSA. Once again, EFSA shows itself to be criminally irresponsible in having no regard for public health issues. If the organization had any scientific credibility at all, it would accept that the Seralini study is cause for great concern; it would ask for a serious reassessment of all existing consents for GM products; and it would ask for a moratorium on all further GMO consents pending a repeat or improvement of the experiments conducted by the Seralini team.

I therefore ask the European Parliament to charge EFSA with conspiring with others to diminish the seriousness of the new research findings -- simply in order to protect its own GMO Panel from charges of serious and systematic scientific assessment failures. Because of these systemic shortcomings, EFSA has consistently favoured the interests of GMO consent applicants while placing the people of Europe increasingly at risk from toxic GM components in the food chain.

Brian John
7th October 2012

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(1) Per Bergman is reported as seeking collaboration with selected civil servants from member states (or selected member states) to discuss scientific concerns and to "avoid divergence". (Minutes, page 2, Teleconference 28 September, organized by EFSA

Emerging Risks Unit). See PDF below, from p 21 onwards.

EFSA documents:
http://www.efsa.europa.eu/en/press/news/121004.htm (4th October 2012)
http://www.efsa.europa.eu/en/efsajournal/pub/2910.htm

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

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ABSTRACT

The health effects of a Roundup-tolerant genetically modified maize (from 11% in the diet), cultivated with or without Roundup, and Roundup alone (from 0.1 ppb in water), were studied 2 years in rats. In females, all treated groups died 2–3 times more than controls, and more rapidly. This difference was visible in 3 male groups fed GMOs. All results were hormone and sex dependent, and the pathological profiles were comparable. Females developed large mammary tumors almost always more often than and before controls, the pituitary was the second most disabled organ; the sex hormonal balance was modified by GMO and Roundup treatments. In treated males, liver congestions and necrosis were 2.5–5.5 times higher. This pathology was confirmed by optic and transmission electron microscopy. Marked and severe kidney nephropathies were also generally 1.3–2.3 greater. Males presented 4 times more large palpable tumors than controls which occurred up to 600 days earlier. Biochemistry data confirmed very significant kidney chronic deficiencies; for all treatments and both sexes, 76% of the altered parameters were kidney related. These results can be explained by the non linear endocrine-disrupting effects of Roundup, but also by the overexpression of the transgene in the GMO and its metabolic consequences.

1. Introduction

There is an ongoing international debate as to the necessary length of mammalian toxicity studies in relation to the consumption of genetically modified (GM) plants including regular metabolic analyses (Séralini et al., 2011). Currently, no regulatory authority requests mandatory chronic animal feeding studies to be performed for edible GMOs and formulated pesticides. However, several studies consisting of 90 day rat feeding trials have been conducted by the biotech industry. These investigations mostly concern GM soy and maize that are rendered either herbicide tolerant (to Roundup (R) in 80% of cases), or engineered to produce a modified *Bt* toxin insecticide, or both. As a result these GM crops contain new pesticide residues for which new maximal residual levels (MRL) have been established in some countries.

If the petitioners conclude in general that there is no major change in genetically modified organism (GMO) subchronic toxicity studies (Domingo and Giné Bordonaba, 2011; Hammond et al., 2004, 2006a,b), significant disturbances have been found and may be interpreted differently (Séralini et al., 2009; Spiroux de Vendômois et al., 2010). Detailed analyses have revealed alterations in kidney and liver functions that may be the signs of early chronic diet intoxication, possibly explained at least in part by pesticide residues in the GM feed (Séralini et al., 2007; Spiroux de Vendômois et al., 2009). Indeed, it has been demonstrated that R concentrations in the range of 10^3 times below the MRL induced endocrine disturbances in human cells (Gasnier et al., 2009) and toxic effects thereafter (Benachour and Seralini, 2009), including *in vivo* (Romano et al., 2012). After several months of consumption of an R-tolerant soy, the liver and pancreas of mice were affected, as highlighted by disturbances in sub-nuclear structure (Malatesta et al., 2008a, 2002a,b). Furthermore, this toxic effect was reproduced by the application of R herbicide directly to hepatocytes in culture (Malatesta et al., 2008b).

Abbreviations: GM, genetically modified; R, Roundup; MRL, maximal residual levels; GMO, genetically modified organism; OECD, Organization for Economic Cooperation and Development; GT, glutamyl-transferase; PCA, principal component analysis; PLS, partial least-squares; OPLS, orthogonal partial least-squares; NIPALS, Nonlinear Iterative Partial Least Squares; OPLS-DA, Orthogonal Partial Least Squares Discriminant Analysis; G, glycogen; L, lipid droplet; N, nucleus; R, rough endoplasmic reticulum (on microscopy pictures only); U, urinary; UEx, excreted in urine during 24 h; APPT, Activated Partial Thromboplastin Time; MCV, Mean Corpuscular Volume; PT, Prothrombine Time; RBC, Red Blood Cells; ALT, alanine aminotransferase; MCHC, Mean Corpuscular Hemoglobin Concentration; A/G, Albumin/Globulin ratio; WBC, White Blood Cells; AST, aspartate aminotransferase.

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Since then, long-term and multi-generational animal feeding trials have been performed with some possibly providing evidence of safety, while others conclude on the necessity of further investigations because of metabolic modifications (Snell et al., 2011). However, none of these studies have included a detailed follow-up of the animals with up to 11 blood and urine samples over 2 years, and none has investigated the NK603 R-tolerant maize.

Furthermore, toxicity evaluation of herbicides is generally performed on mammalian physiology through the long-term study of only their active principle, rather than the formulation used in agriculture, as was the case for glyphosate (Williams et al., 2000), the active herbicide constituent of R. It is important to note that glyphosate is only able to efficiently penetrate target plant organisms with the help of adjuvants present in the various commercially used R formulations (Cox, 2004). When R residues are found in tap water, food or feed, they arise from the total herbicide formulation, which is the most commonly used mixture in agriculture; indeed many authors in the field have strongly emphasized the necessity of studying the potential toxic effects of total chemical mixtures rather than single components (Cox and Surgan, 2006; Mesnage et al., 2010; Monosson, 2005). Even adjuvants and not only glyphosate or other active ingredients are found in ground water (Krogh et al., 2002), and thus an exposure to the diluted whole formulation is more representative of an environmental pollution than the exposure to glyphosate alone in order to study health effects.

With a view to address this lack of information, we have performed a 2 year detailed rat feeding study. The actual guideline 408 of the Organization for Economic Co-operation and Development (OECD) was followed by some manufacturers for GMOs even if it was not designed for that purpose. We have explored more parameters and more frequently than recommended in this standard (Table 1) in a long-term experiment. This allowed us to follow in details potential health effects and their possible origins due to the direct or indirect consequences of the genetic modification itself in GMOs, or due to the formulated herbicide mixture used on GMOs (and not glyphosate alone), or both. Because of recent reviews on GMOs (Domingo and Giné Bordonaba, 2011; Snell et al., 2011) we had no reason to settle at first for a carcinogenesis protocol using 50 rats per group. However we have prolonged the biochemical and hematological measurements or disease status recommended in combined chronic studies using 10 rats per group (up to 12 months in OECD 453). This remains the highest number of rats regularly measured in a standard GMO diet study. We have tested also for the first time 3 doses (rather than two in the usual 90 day long protocols) of the R-tolerant NK603 GM maize alone, the GM maize treated with R, and R alone at very low environmentally relevant doses starting below the range of levels permitted by regulatory authorities in drinking water and in GM feed.

2. Materials and methods

2.1. Ethics

The experimental protocol was conducted in accordance with the regulations of our ethics in an animal care unit authorized by the French Ministries of Agriculture and Research (Agreement Number A35-288-1). Animal experiments were performed according to ethical guidelines of animal experimentations (CEE 86/609 regulation). Concerning field studies of plant species, no specific permits were required, nor for the locations/activities. The maize grown (MON-00603-6 commonly named NK603) was authorized for unconfined release into the environment and use as a livestock feed by the Canadian Food Inspection Agency (Decision Document 2002-35). We confirm that the location is not privately-owned or protected in any way and that the field studies did not involve endangered or protected species. The GM maize was authorized for import into the European Union (CE 258/97 regulation).

2.2. Plants, diets and chemicals

The varieties of maize used in this study were the R-tolerant NK603 (Monsanto Corp., USA), and its nearest isogenic non-transgenic control. These two types of maize were grown under similar normal conditions, in the same location, spaced at a sufficient distance to avoid cross-contamination. The genetic nature, as well as the purity of the GM seeds and harvested material, was confirmed by qPCR analysis of DNA samples. One field of NK603 was treated with R at 3 L ha^{-1} (Weather-MAX, 540 g/L of glyphosate, EPA Reg. 524-537), and another field of NK603 was not treated with R. Corns were harvested when the moisture content was less than 30% and were dried at a temperature below 30 °C. From these three cultivations of

Table 1
Protocol used and comparison to existing assessment, and to non-mandatory regulatory tests.

Treatments and analyses	In this work	Hammond et al., 2004	Regulatory tests
Treatments + controls	GMO NK603, GMO NK603 + Roundup, Roundup, and closest isogenic maize	GMO NK603 + Roundup, closest isogenic maize, and six other maize lines non substantially equivalent	GMOs or chemicals (in standard diet or water)
Doses by treatment	3	2	At least 3
Duration in months	24 (chronic)	3 (subchronic: 13 weeks)	3
Animals measured/group/sex	10/10 SD rats (200 rats measured)	10/20 SD rats (200 rats measured/total 400)	At least 10 rodents
Animals by cage (same sex)	1–2	1	1 or more
Monitoring/week	2	1	1 or more
Feed and water consumptions	Measured	For feed only	At least feed
Organs and tissues studied			For high dose and controls
Histology/animal	34	17/36	At least 30
Organs weighted	10	7	At least 8
Electronic microscopy	Yes	No	No
Behavioral studies (times)	2	1 (no protocol given)	1
Ophtalmology (times)	2	0	2
Number of blood samples/animal	11, each month (0–3) then every 3 months	2, weeks 4 and 13	1, at the end
Blood parameters	31 (11 times for most)	31 (2 times)	At least 25 (at least 2 times)
Plasma sex steroids	Testosterone, estradiol	No	No, except if endocrine effects suspected
Liver tissue parameters	6	0	0
Number of urine samples	11	2	Optional, last week
Urine parameters studied	16	18	7 if performed
Microbiology in feces or urine	Yes	Yes	No
Roundup residues in tissues	Studied	Not studied	Not mandatory
Transgene in tissues	Studied	Not studied	Not studied

The protocol used in this work was compared to the regulatory assessment of NK603 maize by the company (Hammond et al., 2004), and to non mandatory regulatory *in vivo* tests for GMOs, or mandatory for chemicals (OECD 408). Most relevant results are shown in this paper.

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maize, laboratory rat chow was made based on the standard diet A04 (Safe, France). The dry rat feed was made to contain 11, 22 or 33% of GM maize, cultivated either with or without R, or 33% of the non-transgenic control line. The concentrations of the transgene were confirmed in the three doses of each diet by qPCR. All feed formulations consisted in balanced diets, chemically measured as substantially equivalent except for the transgene, with no contaminating pesticides over standard limits. All secondary metabolites cannot be known and measured in the composition. However we have measured isoflavones and phenolic acids including ferulic acid by standard HPLC-UV. All reagents used were of analytical grade. The herbicide diluted in the drinking water was the commercial formulation of R (GT Plus, 450 g/L of glyphosate, approval 2020448, Monsanto, Belgium). Herbicides levels were assessed by glyphosate measurements in the different dilutions by mass spectrometry.

2.3. Animals and treatments

Virgin albino Sprague-Dawley rats at 5 weeks of age were obtained from Harlan (Gannat, France). All animals were kept in polycarbonate cages (820 cm^2, Genestil, France) with two animals of the same sex per cage. The litter (Toplit classic, Safe, France) was replaced twice weekly. The animals were maintained at 22 ± 3 °C under controlled humidity (45–65%) and air purity with a 12 h-light/dark cycle, with free access to food and water. The location of each cage within the experimental room was regularly moved. This 2 year life-long experiment was conducted in a GPL environment according to OECD guidelines. After 20 days of acclimatization, 100 male and 100 female animals were randomly assigned on a weight basis into 10 equivalent groups. For each sex, one control group had access to plain water and standard diet from the closest isogenic non-transgenic maize control; six groups were fed with 11, 22 and 33% of GM NK603 maize either treated or not with R. The final three groups were fed with the control diet and had access to water supplemented with respectively 1.1×10^{-8}% of R (0.1 ppb of R or 50 ng/L of glyphosate, the contaminating level of some regular tap waters), 0.09% of R (400 mg/kg, US MRL of glyphosate in some GM feed) and 0.5% of R (2.25 g/L, half of the minimal agricultural working dilution). This was changed weekly. Twice weekly monitoring allowed careful observation and palpation of animals, recording of clinical signs, measurement of any tumors that may arise, food and water consumption, and individual body weights.

2.4. Biochemical analyses

Blood samples were collected from the tail vein of each rat under short isoflurane anesthesia before treatment and after 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 months: 11 measurements were obtained for each animal alive at 2-years. It was first demonstrated that anesthesia did not impact animal health. Two aliquots of plasma and serum were prepared and stored at −80° C. Then 31 parameters were assessed (Table 1) according to standard methods including hematology and coagulation parameters, albumin, globulin, total protein concentration, creatinine, urea, calcium, sodium, potassium, chloride, inorganic phosphorus, triglycerides, glucose, total cholesterol, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl-transferase (GT), estradiol, testosterone. In addition, at months 12 and 24 the C-reactive protein was assayed. Urine samples were collected similarly 11 times, over 24 h in individual metabolic cages, and 16 parameters were quantified including creatinine, phosphorus, potassium, chloride, sodium, calcium, pH and clairance. Liver samples at the end made it possible to perform assays of CYP1A1, 1A2, 3A4, 2C9 activities in S9 fractions, with glutathione S- transferase and gamma-GT.

2.5. Anatomopathology

Animals were sacrificed during the course of the study only if necessary because of suffering according to ethical rules (such as 25% body weight loss, tumors over 25% body weight, hemorrhagic bleeding, or prostration), and at the end of the study by exsanguination under isoflurane anesthesia. In each case, the following organs were collected: brain, colon, heart, kidneys, liver, lungs, ovaries, spleen, testes, adrenals, epididymis, prostate, thymus, uterus, aorta, bladder, bone, duodenum, esophagus, eyes, ileum, jejunum, lymph nodes, lymphoreticular system, mammary glands, pancreas, parathyroid glands, Peyer's patches, pituitary, salivary glands, sciatic nerve, skin, spinal cord, stomach, thyroid and trachea. The first 14 organs (at least 10 per animal depending on the sex, Table 1) were weighted, plus any tumor that arose. The first nine organs were divided into two parts and one half was immediately frozen in liquid nitrogen/carbonic ice. The remaining parts including other organs were rinsed in PBS and stored in 4% formalin before anatomopathological study. These samples were used for further paraffin-embedding, slides and HES histological staining. For transmission electron microscopy, kidneys, livers and tumors were cut into 1 mm^3 fragments. Samples were fixed in pre-chilled 2% paraformaldehyde/2.5% glutaraldehyde in 0.1 M PBS pH 7.4 at 4 °C for 3 h and processed as previously described (Malatesta et al., 2002a).

2.6. Statistical analysis

Biochemical data were treated by multivariate analysis with the SIMCA-P (V12) software (UMETRICS AB Umea, Sweden). The use of chemometrics tools, for example, principal component analysis (PCA), partial least-squares to latent structures (PLS), and orthogonal PLS (OPLS), are robust methods for modeling, analyzing and interpreting complex chemical and biological data. OPLS is a recent modification of the PLS method. PLS is a regression method used in order to find the relationship between two data tables referred to as X and Y. PLS regression (Eriksson et al., 2006b) analysis consists in calculating by means of successive iterations, linear combinations of the measured X-variables (predictor variables). These linear combinations of X-variables give PLS components (score vectors t). A PLS component can be thought of as a new variable – a latent variable – reflecting the information in the original X-variables that is of relevance for modeling and predicting the response Y-variable by means of the maximization of the square of covariance (Max $cov^2(X,Y)$). The number of components is determined by cross validation. SIMCA software uses the Nonlinear Iterative Partial Least Squares algorithm (NIPALS) for the PLS regression. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was used in this study (Weljie et al., 2011; Wiklund et al., 2008). The purpose of Discriminant Analysis is to find a model that separates groups of observations on the basis of their X variables. The X matrix consists of the biochemical data. The Y matrix contains dummy variables which describe the group membership of each observation. Binary variables are used in order to encode a group identity. Discriminant analysis finds a discriminant plan in which the projected observations are well separated according to each group. The objective of OPLS is to divide the systematic variation in the X-block into two model parts, one linearly related to Y (in the case of a discriminant analysis, the group membership), and the other one unrelated (orthogonal) to Y. Components related to Y are called predictive, and those unrelated to Y are called orthogonal. This partitioning of the X data results in improved model transparency and interpretability (Eriksson et al., 2006a). Prior to analysis, variables were mean-centered and unit variance scaled.

3. Results

3.1. Mortality

Control male animals survived on average 624 ± 21 days, whilst females lived for 701 ± 20, during the experiment, plus in each case 5 weeks of age at the beginning and 3 weeks of stabilization period. After mean survival time had elapsed, any deaths that occurred were considered to be largely due to aging. Before this period, 30% control males (three in total) and 20% females (only two) died spontaneously, while up to 50% males and 70% females died in some groups on diets containing the GM maize (Fig. 1). However, the rate of mortality was not proportional to the treatment dose, reaching a threshold at the lowest (11%) or intermediate (22%) amounts of GM maize in the equilibrated diet, with or without the R application on the plant. It is noteworthy that the first two male rats that died in both GM treated groups had to be euthanized due to kidney Wilm's tumors that were over 25% of body weight. This was at approximately a year before the first control animal died. The first female death occurred in the 22% GM maize feeding group and resulted from a mammary fibroadenoma 246 days before the first control. The maximum difference in males was 5 times more deaths occurring during the 17th month in the group consuming 11% GM maize, and in females 6 times greater mortality during the 21st month on the 22% GM maize diet with and without R. In the female cohorts, there were 2–3 times more deaths in all treated groups compared to controls by the end of the experiment and earlier in general. Females were more sensitive to the presence of R in drinking water than males, as evidenced by a shorter lifespan. The general causes of death represented in histogram format (Fig. 1) are linked mostly to large mammary tumors in females, and other organic problems in males.

3.2. Anatomopathological observations

All rats were carefully monitored for behavior, appearance, palpable tumors, infections, during the experiment, and at least 10 organs per animal were weighted and up to 34 analyzed post mortem, at the macroscopic and/or microscopic levels (Table 1).

Please cite this article in press as: Séralini, G.-E., et al. Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food Chem. Toxicol. (2012), http://dx.doi.org/10.1016/j.fct.2012.08.005

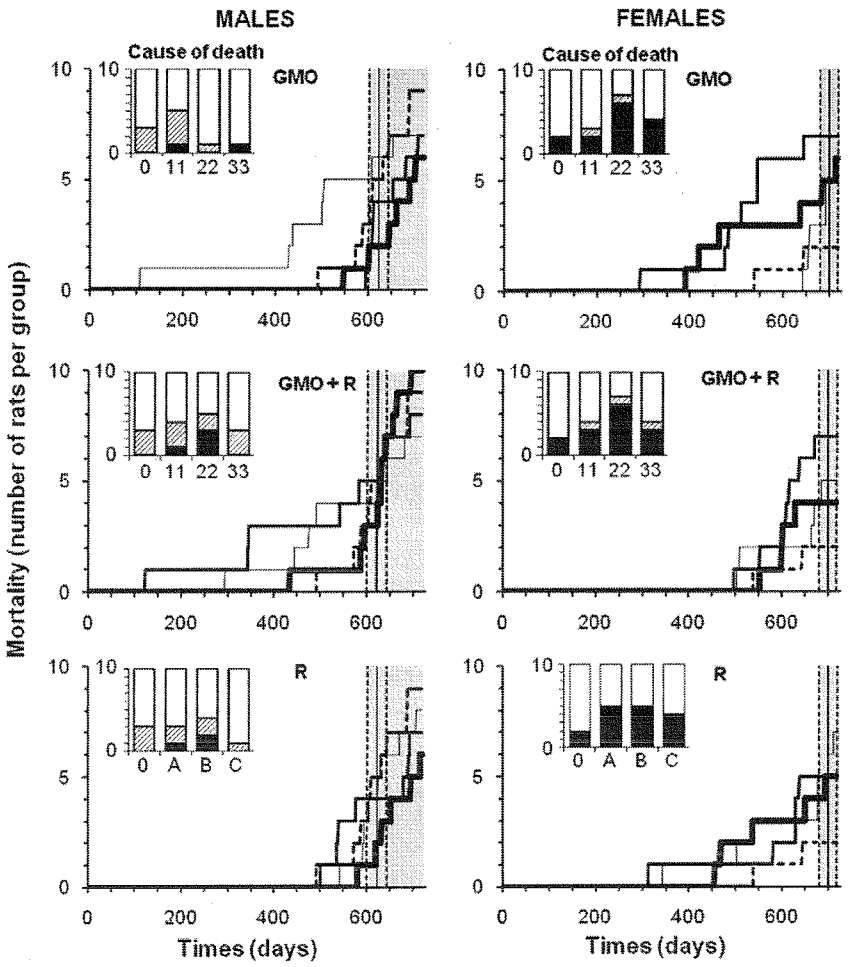

Fig. 1. Mortality of rats fed GMO treated or not with Roundup, and effects of Roundup alone. Rats were fed with NK603 GM maize (with or without application of Roundup) at three different doses (11, 22, 33% in their diet: thin, medium and bold lines, respectively) compared to the substantially equivalent closest isogenic non-GM maize (control, dotted line). Roundup was administrated in drinking water at 3 increasing doses, same symbols (environmental (A), MRL in agricultural GMOs (B) and half of minimal agricultural levels (C), see Section 2). Lifespan during the experiment for the control group is represented by the vertical bar ± SEM (grey area). In bar histograms, the causes of mortality before the grey area are detailed in comparison to the controls (0). In black are represented the necessary euthanasia because of suffering in accordance with ethical rules (tumors over 25% body weight, more than 25% weight loss, hemorrhagic bleeding, etc.); and in hatched areas, spontaneous mortality.

All data cannot be shown in one report, and the most relevant are described here. There was no rejection by the animals of the diet with or without GMOs, nor any major difference in the body weight.

The largest palpable growths (above a diameter of 17.5 mm in females and 20 mm in males) were found to be in 95% of cases non-regressive tumors, and were not infectious nodules. These growths progressively increased in size and number, but not proportionally to the treatment dose over the course of the experiment (Fig. 2). As in the case of rates of mortality, this suggests that a threshold in effect was reached at the lowest doses. They were rarely equal but almost always more frequent than in controls for all treated groups, often 2–3 times more in both sexes. Tumors began to reach a large size on average 94 days before in treated females, and up to 600 days earlier in 2 male groups eating the GM maize (11 and 22% with or without R).

In female animals, the largest tumors were in total 5 times more frequent than in males after 2 years, with 93% being mammary tumors. Adenomas, fibroadenomas and carcinomas were deleterious to health due to a very large size, rather than the grade of the tumor itself. Large tumor size caused impediments to either breathing or nutrition and digestion because of their thoracic or abdominal location and also resulted in hemorrhaging. In addition, one metastatic ovarian cystadenocarcinoma and two skin tumors were identified. Metastases were observed in only 2 cases; one in a group fed with 11% GM maize, and another in the highest dose of R treatment group.

Up to 14 months, no animals in the control groups showed any signs of tumors whilst 10–30% of treated females per group developed tumors, with the exception of one group (33% GMO + R). By the beginning of the 24th month, 50–80% of female animals had developed tumors in all treated groups, with up to 3 tumors per animal, whereas only 30% of controls were affected. The R treatment groups showed the greatest rates of tumor incidence with 80% of animals affected with up to 3 tumors for one female, in each group. A summary of all mammary tumors at the end of the experiment, independent of the size, is presented in Table 2. The same trend was observed in the groups receiving R in their drinking water; all females except one (with metastatic ovarian carcinoma) presented, in addition mammary hypertrophies and in some cases hyperplasia with atypia (Table 2).

The second most affected organ in females was the pituitary gland, in general around 2 times more than in controls for most treatments (Table 2). At this level again, adenomas and/or hyper-

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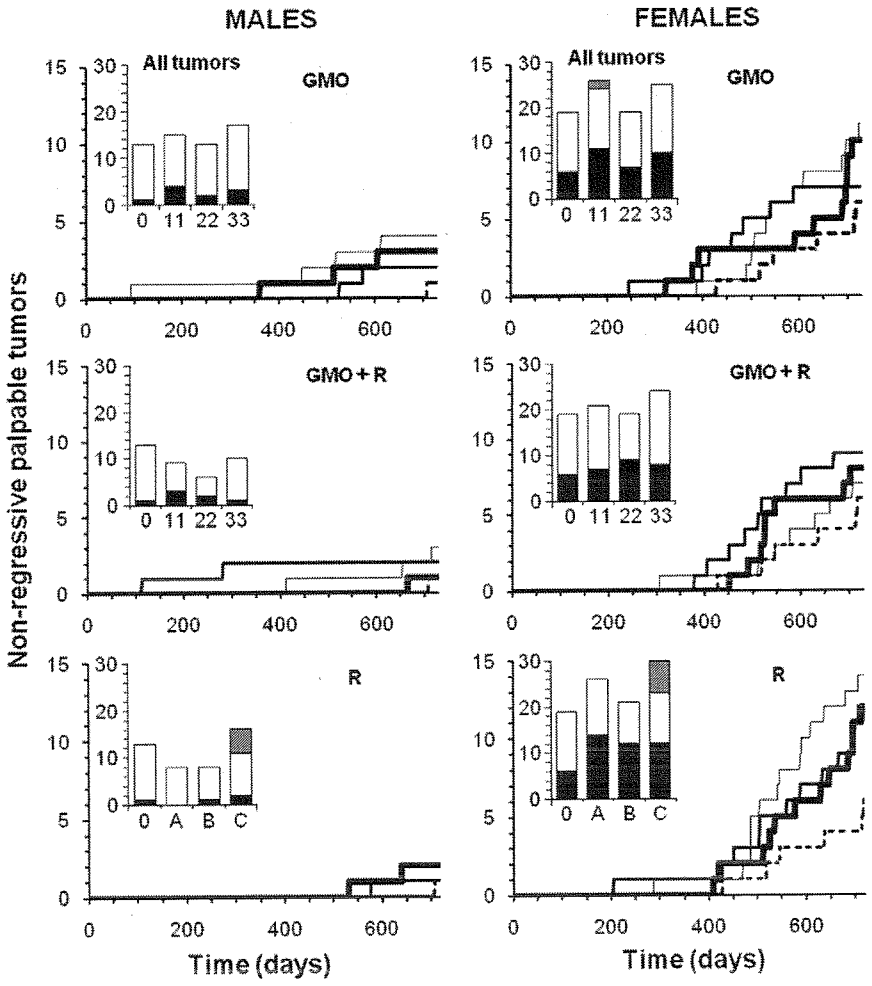

Fig. 2. Largest non-regressive tumors in rats fed GMO treated or not by Roundup, and effects of Roundup alone. The symbols of curves and treatments are explained in the caption of Fig. 1. The largest tumors were palpable during the experiment and numbered from 20 mm in diameter for males and 17.5 mm for females. Above this size, 95% of growths were non-regressive tumors. Summary of all tumors are shown in the bar histograms: black, non regressive largest tumors; white, small internal tumors; grey, metastases.

Table 2
Summary of the most frequent anatomical pathologies observed.

Organs and associated pathologies	Controls	GMO 11%	GMO 22%	GMO 33%	GMO 11% + R	GMO 22% + R	GMO 33% + R	R (A)	R (B)	R (C)
Males, in liver	2 (2)	5 (4)	11 (7)	8 (6)	5 (4)	7 (4)	6 (5)	11 (5)	9 (7)	6 (5)
In hepatodigestive tract	6 (5)	10 (6)	13 (7)	9 (6)	9 (6)	13 (6)	11 (7)	23 (9)	16 (8)	9 (5)
Kidneys, CPN	3 (3)	4 (4)	5 (5)	7 (7)	5 (5)	4 (4)	4 (4)	6 (6)	5 (5)	3 (3)
Females, mammary tumors	8 (5)	15 (7)	10 (7)	15 (8)	10 (6)	11 (7)	13 (9)	20 (9)	16 (10)	12 (9)
In mammary glands	10 (5)	22 (8)	10 (7)	16 (8)	17 (8)	16 (8)	15 (9)	26 (10)	20 (10)	18 (9)
Pituitary	9 (6)	23 (9)	20 (8)	8 (5)	19 (9)	9 (4)	19 (7)	22 (8)	16 (7)	13 (7)

After the number of pathological abnormalities, the number of rats reached is indicated in parentheses. In male animals pathological signs are liver congestions, macroscopic spots and microscopic necrotic foci. Hepatodigestive pathological signs concern the liver, stomach and small intestine (duodenum, ileum or jejunum). Only marked or severe chronic progressive nephropathies (CPN) are listed, excluding two nephroblastomas in groups consuming GMO 11% and GMO 22% + Roundup. In females, mammary fibroadenomas and adenocarcinomas are the major tumors detected; galactoceles and hyperplasias with atypia are also found and added in mammary glands pathological signs. Pituitary dysfunctions include adenomas, hyperplasias and hypertrophies. For details of the various treatment groups see Fig. 1.

plasias and hypertrophies were noticed. For all R treatment groups, 70–80% of animals presented 1.4–2.4 times more abnormalities than controls in this gland.

The big palpable tumors in males (in kidney, and mostly skin) were by the end of the experimental period on average twice as frequent as in controls, in which one skin fibroma appeared during the 23rd month. At the end of the experiment, internal non-palpable tumors were added, and their sums were lower in males than in females. They were not really different from controls, although slightly above in females (Histograms Fig. 2).

The most affected organs in males were the liver, together with the hepatodigestive tract and kidneys (Table 2 and Fig. 3). Hepatic congestions, macroscopic and microscopic necrotic foci were 2.5–5.5 times more frequent in all treatments than in control groups. Gamma GT hepatic activity was increased in particular for GMO + R groups (up to 5.4 times), this being probably due to a liver disorder.

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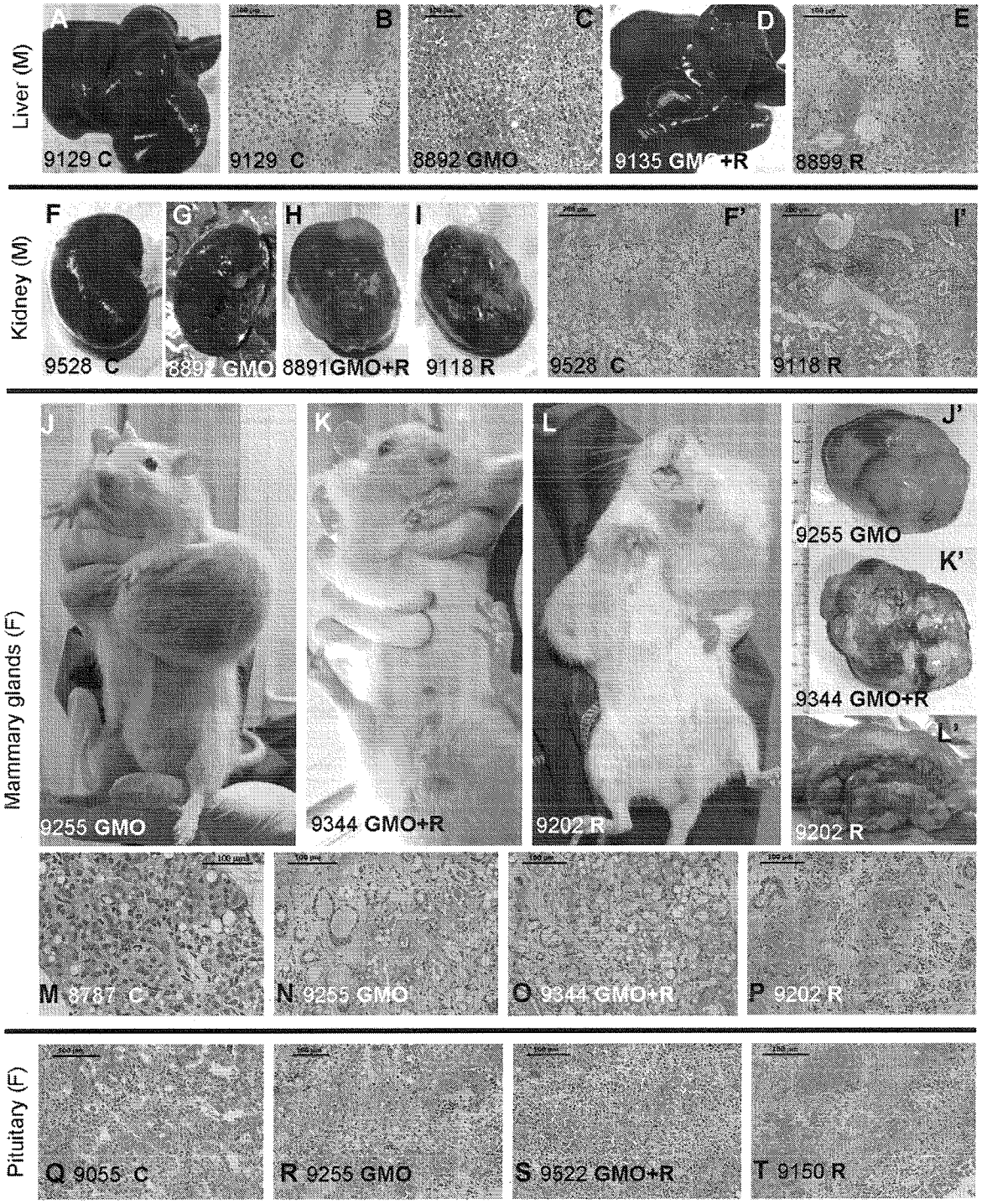

Fig. 3. Anatomopathological observations in rats fed GMO treated or not by Roundup, and effects of Roundup alone. Macroscopic and microscopic photographs show male livers (A–E) and left kidneys (F–I'), female mammary glands (J–P) and pituitaries (Q–T), according to Table 2. The number of each animal and its treatment is specified. Macroscopic pale spots (D) and microscopic necrotic foci in liver (C clear-cell focus, E basophilic focus with atypia), and marked or severe chronic progressive nephropathies, are illustrated. In females, mammary tumors (J,J',N adenocarcinoma and K,K',L,L',O,P fibroadenomas) and pituitary adenomas (R–T) are shown and compared to controls (C after the rat number).

In addition, cytochrome activities also generally increased in the presence of R (in drinking water or GM diet) according to the dose up to 5.7 times at the highest dose. Transmission electron microscopic observations of liver samples confirmed changes for all treated groups in relation to glycogen dispersion or appearance in lakes, increase of residual bodies and enlargement of cristae in mitochondria (Fig. 4). The GM maize fed groups either with or without R application (in plants) showed a reduced transcription in mRNA and rRNA because of higher heterochromatin content, and decreased nucleolar dense fibrillar components. In the GMO + R group (at the highest dose) the smooth endoplasmic reticulum was drastically increased and nucleoli decreased in size,

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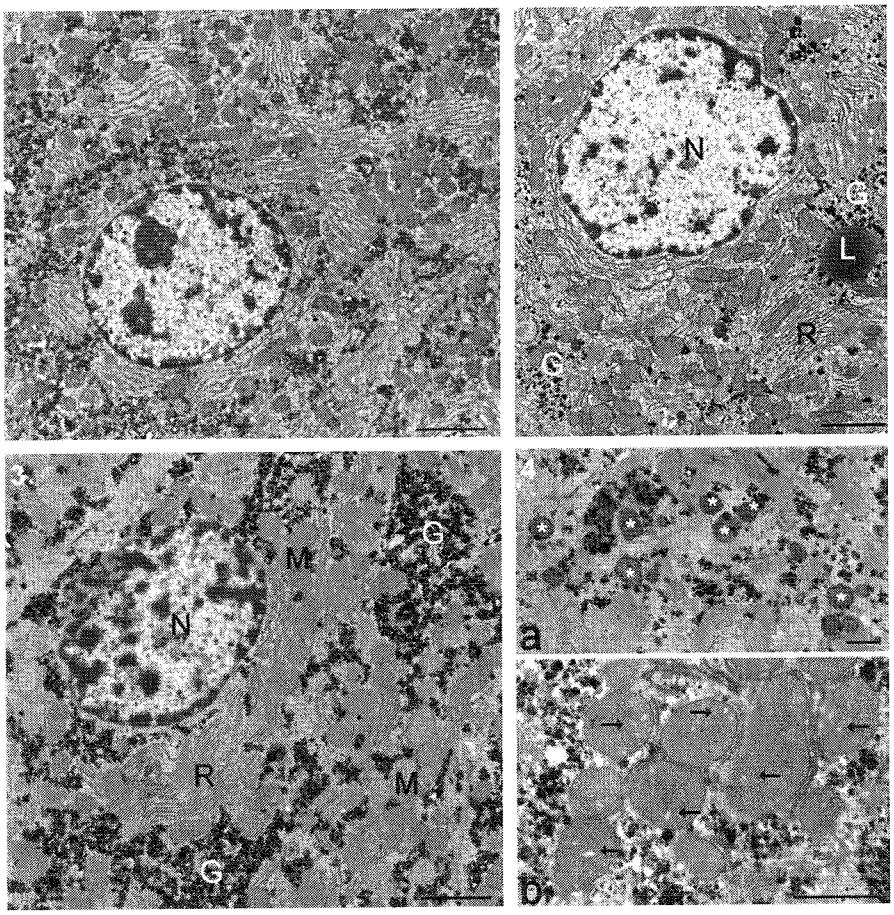

Fig. 4. Ultrastructure of hepatocytes in male rats from groups presenting the greatest degree of liver pathology. (1) Typical control rat hepatocyte (Bar 2 μm except in 4). (2) Effects with Roundup at the lowest dose. Glycogen (G) is dispersed in the cytoplasm. L, lipid droplet; N, nucleus; R rough endoplasmic reticulum. (3) Hepatocytes of animal fed GM maize (GMO) at 22% of total diet. Large lakes of glycogen occur in the cytoplasm. M, mitochondria. (4) Details of treatment effects with 22% dietary GMO (Bar 1 μm). (a) Cluster of residual bodies (asterisks). (b) Mitochondria show many enlarged cristae (arrows).

becoming more compact. For R treatment alone similar trends were observed, with a partial resumption of nucleolar activity at the highest dose.

Degenerating kidneys with turgid inflammatory areas demonstrate the increased incidence of marked and severe chronic progressive nephropathies. which were up to 2-fold higher in the 33% GM maize or lowest dose R treatment groups (Table 2 and Fig. 3).

3.3. Biochemical analyses

For the different corns and diets, the study of the standard chemical composition revealed no particular difference; this is why they were classified as substantially equivalent, except for transgene DNA quantification. For instance, there was no difference between total isoflavones. In addition, other specific compounds not always requested for substantial equivalence establishment were assayed. Among phenolic acids, the only consistent and significant ($p < 0.01$) results concerned ferulic acid that was decreased in both GM and GM + R diets by 16–30% in comparison to the control diet (889 ± 107, 735 ± 89 respectively vs control 1057 ± 127 mg/kg) and caffeic acid by 21–53% (17.5 ± 2.1, 10.3 ± 1.3 vs control 22.1 ± 2.6 mg/kg).

For biochemical measurements in rats, statistical analysis was performed on the results obtained from samples taken at the 15th month time point, as this was the last sampling time when most animals were still alive (in treated groups 90% males, 94% females, and 100% controls). OPLS-DA 2-class models were built between each treated group per sex and controls. Only models with an explained variance $R^2(Y) \geqslant 80\%$, and a cross-validated predictive ability $Q^2(Y) \geqslant 60\%$, were used for selection of the discriminant variables (Fig. 5A), when their regression coefficients were significant at 99% confidence level. Thus, in treated females, kidney failures appeared at the biochemical level (82% of the total disrupted parameters). Ions (Na, Cl) or urea increased in urine. Accordingly, the same ions decreased in serum (Fig. 5B) as did the levels of P, K and Ca. Creatinine or clairance decreased in urine for all treatment groups in comparison to female controls (Table 3). In GM maize treated males (with or without R), 87% of discriminant variables were kidney related, but the disrupted profiles were less obvious because of advanced chronic nephropathies and deaths. In summary, for all treatments and both sexes, 76% of the discriminant variables versus controls were kidney related.

Moreover, in females (Table 3) the androgen/estrogen balance in serum was modified by GM maize and R treatments (at least 95% confidence level, Fig. 5B), and for male animals at the highest R-treatment dose, levels of estrogens were more than doubled.

4. Discussion

This report describes the first life-long rodent (rat) feeding study investigating possible toxic effects rising from an R-tolerant

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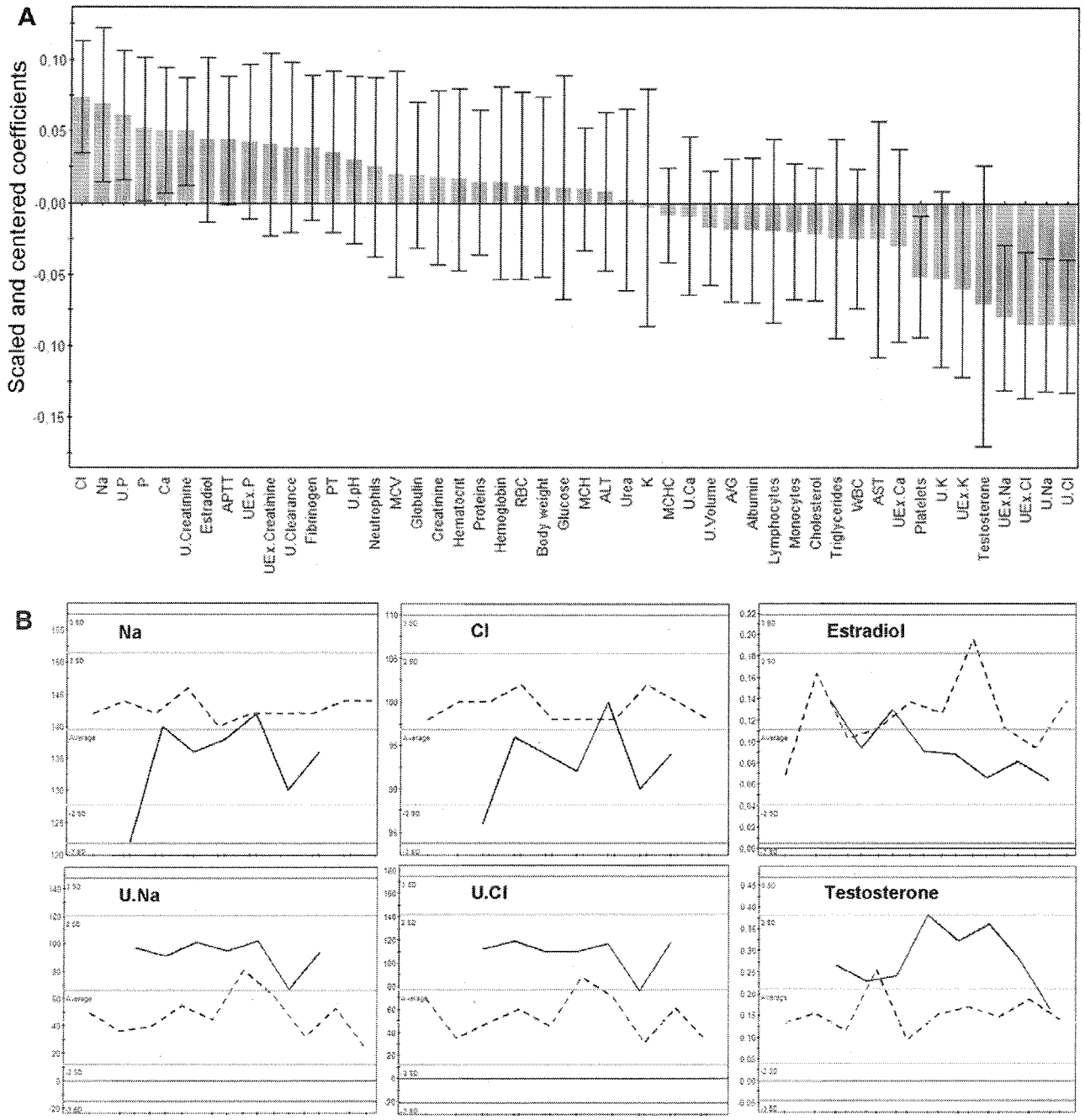

Fig. 5. Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) for biochemical data (females fed 33% GMO versus controls). (A) OPLS-DA regression coefficients for predictive component, with jack-knifed confidence intervals at 99% confidence level, indicate discriminant parameters versus controls at month 15 (Abbreviations: U Urinary, UEx Excreted in urine during 24 h, APPT Activated Partial Thromboplastin Time, MCV Mean Corpuscular Volume, PT Prothrombine Time, RBC Red Blood Cells, ALT ALanine aminoTransferase, MCHC Mean Corpuscular Hemoglobin Concentration, A/G Albumin/Globulin ratio, WBC White Blood Cells, AST aspartate aminotransferase). (B) In this case, detailed examples of significant discriminant variables distribution between females fed 33% GMO (bold line) and controls (dotted line). On *x* axis: animals; on *y* axis: serum or urine biochemical values for Na, Cl, estradiol, testosterone. Profiles evidence kidney ion leakages and sex hormonal imbalance versus controls.

GM maize (NK603) and a complete commercial formulation of R-herbicide.

Our data show 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 R application; R alone), non-monotonic and with a threshold effect (Vandenberg et al., 2012). Similar degrees of pathological symptoms were noticed in this study to occur from the lowest to the highest doses suggesting a threshold effect. This corresponds to levels likely to arise from consumption or environmental exposure, such as either 11% GM maize in food, or 50 ng/L of glyphosate in R-formulation as can be found in some contaminated drinking tap waters, and which fall within authorized limits.

The lifespan of the control group of animals corresponded to the mean rat lifespan, but as is frequently the case with most mammals including humans (WHO, 2012), males on average died before females, except for some female treatment groups. All treatments in both sexes enhanced large tumor incidence by 2–3-fold in com-

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Table 3
Percentage variation of parameters indicating kidney failures of female animals.

Discriminant variables		GMO 11% + R	GMO 22% + R	GMO 33% + R	GMO 11%	GMO 22%	GMO 33%	R (A)	R (B)	R (C)
Urinary decrease	Clairance	−4	−11	−20	**−20**	**−20**	−19	**−20**	**−24**	**−40**
	Creatinine	−5	**−32**	**−37**	−19	−37	**−36**	−43	−23	−1
	Creatinine ex	−5	−11	**−19**	−18	**−17**	−21	**−21**	**−22**	**−39**
Urinary increase	Urea	12	**18**	15	15	12	−1	0	13	**32**
	Na	25	33	30	52	−2	**95**	**62**	65	**91**
	Na ex	24	50	68	50	24	**125**	**108**	51	7
	Cl	14	35	28	46	5	**101**	**67**	56	**94**
	Cl ex	20	63	70	51	31	**138**	**121**	48	13
Serum decrease	Na	2	1	1	−1	**−4**	**−6**	−7	0	−3
	Cl	−1	−2	−2	−5	−7	**−6**	**−8**	−1	−4
	P	−6	−11	−13	−17	**−18**	**−20**	**−32**	−9	−13
	K	4	5	10	2	−4	0	−4	8	**−5**
	Ca	4	3	3	**2**	−2	**−5**	−6	3	**−6**
Gonads	Estradiol	8	−1	2	5	−2	−25	−26	**−73**	39
	Testosterone	5	−9	27	**56**	17	81	**97**	**−72**	10

OPLS-DA was performed on 48 variables at month 15. Here we showed mean differences (%) of variables (discriminant at 99% confidence level, in bold character) indicating kidney parameters of female animals, together with sex hormones. Male kidney pathologies are already illustrated in Table 2.

parison to our controls but also for the number of mammary tumors in comparison to the same Harlan Sprague Dawley strain (Brix et al., 2005), and overall around 3-fold in comparison to the largest study with 1329 Sprague Dawley female rats (Chandra et al., 1992). In our study the tumors also developed considerably faster than the controls, even though the majority of tumors were observed after 18 months. The first large detectable tumors occurred at 4 and 7 months into the study in males and females respectively, underlining the inadequacy of the standard 90 day feeding trials for evaluating GM crop and food toxicity (Séralini et al., 2011).

Suffering inducing euthanasia and deaths corresponded mostly in females to the development of large mammary tumors. These appeared to be clearly related to the various treatments when compared to the control groups. These tumors are generally known to be mostly estrogen-dependent (Harvell et al., 2000). We observed a strikingly marked induction of mammary tumors by R alone, a major formulated pesticide, even at the very lowest dose administered. R has been shown to disrupt aromatase which synthesizes estrogens (Richard et al., 2005), but to also interfere with estrogen and androgen receptors in cells (Gasnier et al., 2009). In addition, R appears to be a sex endocrine disruptor *in vivo*, also in males (Romano et al., 2010). Sex steroids are also modified in treated rats. These hormone-dependent phenomena are confirmed by enhanced pituitary dysfunction in treated females. An estrogen modified feedback mechanism may act at this level (Popovics et al., 2011; Walf and Frye, 2010). The similar pathological profiles provoked by the GM maize containing R residues may thus be explained at least by R residues themselves, knowing that the medium dose of the R treatment corresponds to acceptable levels of this pesticide residues in GMOs.

Interestingly, in the groups of animals fed with the NK603 without R application, similar effects with respect to enhanced tumor incidence and mortality rates were observed. A possible explanation for this finding is the production of specific compound(s) in the GM feed that are either directly toxic and/or cause the inhibition of pathways that in turn generate chronic toxic effects. This is despite the fact that the variety of GM maize used is this study was judged by industry and regulators as being substantially equivalent to the corresponding non-GM closest isogenic line. As the total chemical composition of the GM maize cannot be measured in details, the use of substantial equivalence is insufficient to highlight potential unknown toxins and therefore cannot replace long-term animal feeding trials for GMOs. A cause of the effects of the effects could be that the NK603 GM maize used in this study is engineered to overexpress a modified version of the *Agrobacterium tumefaciens* 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Hammond et al., 2004) allowing the R tolerance. The modified EPSPS is not inhibited by glyphosate by contrast to the wild enzyme. This enzyme is known to drive the first step of aromatic amino acid biosynthesis in the plant shikimate pathway; in addition estrogenic isoflavones and their glycosides are also products of this pathway (Duke et al., 2003). They were not disturbed in our study. By contrast, the levels of caffeic and ferulic acids in the GM diets, which are also secondary metabolites from this pathway, but not always measured in regulatory tests, are significantly reduced. This may lower their protective effects against carcinogenesis and even mammalian tumors (Kuenzig et al., 1984; Baskaran et al., 2010). Moreover, these phenolic acids and in particular ferulic acid may modulate estrogen receptors or the estrogenic pathway in mammalian cells (Chang et al., 2006). This does not exclude the action of other unknown metabolites. This explanation also corresponds to the fact that the observed effects of NK603 and R are not additive and reached a threshold. This implies that both the NK603 maize and R may cause hormonal disturbances in the same biochemical and physiological pathway.

As expected, mammary tumors in males occurred far less frequently than in females. Death in male rats was mostly due to the development of severe hepatorenal insufficiencies, confirming the first signs of toxicity observed in 90 day feeding trials with NK603 maize (Spiroux de Vendômois et al., 2009). In females, kidney ion leakages were evidenced at the biochemical levels at month 15, when severe nephropathies were evidenced in dead male animals afterwards, at the anatomopathological level. Early signs of toxicity at month 3 in kidney and liver were also observed for 19 edible GM crops containing pesticide residues (Séralini et al., 2011). As a matter of fact, only elderly male rats are sensitive to chronic progressive nephropathies (Hard and Khan, 2004). The disturbed kidney parameters may have been induced by the reduction of phenolic acids in our study, since caffeic and ferulic acids are beneficial in the kidney as they prevent oxidative stress (Srinivasan et al., 2005; U Rehman and Sultana, 2011). Accordingly, we previously demonstrated that plant extracts containing ferulic and caffeic acids were able to promote detoxification of embryonic kidney cells after R contamination (Gasnier et al., 2011). It is thus possible that NK603 consumption by reducing these compounds may well provoke an early aging of kidney physiology in this study, like R by oxidative stress.

Disturbances that we found to occur in the male liver are characteristic of a chronic intoxication, confirmed by alterations

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in biochemical liver and kidney function parameters. The observation that liver function in female animals is less affected may be due to their physiology being better adapted to estrogen metabolism. Furthermore, liver enzymes have been clearly demonstrated as sex-specific in their expression patterns, including in a 90-day rat feeding trial of NK603 maize (Spiroux de Vendômois et al., 2009). However, in a long-term study, evidence of early liver aging was observed in female mice fed with R-tolerant GM soy (Malatesta et al., 2008a). In the present investigation, deeper analysis at an ultrastructural level revealed evidence of impediments in transcription and other defects in cell nuclear structure that were comparable in both sexes, and dose-dependent in hepatocytes in all treatments. This is consistent with the well-documented toxic effect of very low dilutions of R on apoptosis, mitochondrial function, and cell membrane degradation inducing necrosis of hepatocytes, and other cell lines (Benachour and Seralini, 2009; Benachour et al., 2007; Gasnier et al., 2010; Peixoto, 2005).

The disruptions of at least the estrogen-related pathways and/or enhancement of oxidative stress by all treatments need further investigations. This can be addressed through the application of transcriptomic, proteomic and metabolomic methods to analyze the molecular profiles of kidneys and livers, as well as the GM NK603 maize (Jiao et al., 2010; Zhou et al., 2009; Zolla et al., 2008). Other possible causes of observed pathogenic effects may be due to disturbed gene expression resulting from the transgene insertional, general mutagenic or metabolic effects (Latham et al., 2006; Wilson et al., 2006) as has been shown for MON810 GM maize (Rosati et al., 2008). A consequent disruption of general metabolism in the GMO cannot be excluded, which could lead, for example, to the production of other potentially active compounds such as miRNAs (Zhang et al., 2012) or leukotoxin diols (Markaverich et al., 2005).

In conclusion, it was previously known that glyphosate consumption in water above authorized limits may provoke hepatic and kidney failures (EPA). The results of the study presented here clearly demonstrate that lower levels of complete agricultural glyphosate herbicide formulations, at concentrations well below officially set safety limits, induce severe hormone-dependent mammary, hepatic and kidney disturbances. Similarly, disruption of biosynthetic pathways that may result from overexpression of the EPSPS transgene in the GM NK603 maize can give rise to comparable pathologies that may be linked to abnormal or unbalanced phenolic acids metabolites, or related compounds. Other mutagenic and metabolic effects of the edible GMO cannot be excluded. This will be the subject of future studies, including transgene and glyphosate presence in rat tissues. Reproductive and multigenerational studies will also provide novel insights into these problems. This study represents the first detailed documentation of long-term deleterious effects arising from the consumption of a GM R-tolerant maize and of R, the most used herbicide worldwide.

Altogether, the significant biochemical disturbances and physiological failures documented in this work confirm the pathological effects of these GMO and R treatments in both sexes, with different amplitudes. We propose that agricultural edible GMOs and formulated pesticides must be evaluated very carefully by long term studies to measure their potential toxic effects.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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European Food Safety Authority

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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]

European Food Safety Authority (EFSA), Parma, Italy

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

[1] On request from European Commission Question No EFSA-Q-2012-00841, approved on 3 October 2012.

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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) (1.1×10^{-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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Advies van de directeur bureau Risicobeoordeling en onderzoeksprogramming (BuRO) over de risicobeoordeling van het artikel Séralini et al. (2012)

Datum
01 oktober 2012

ADVIES

Wetenschappelijke risicobeoordeling van de publicatie van Séralini en coauteurs over gezondheidsrisico's voor mens en dier na vervoederen van Roundup-tolerante GM-maïs en Roundup herbicide aan ratten

Achtergrond

Op 19 september 2012 heeft het wetenschappelijk tijdschrift Food and Chemical Toxicology een artikel openbaar gemaakt van Séralini en coauteurs over de mogelijke schadelijke effecten van een bepaalde variant van genetisch gemodificeerde maïs (NK603) en het herbicide Roundup (waarvan het hoofdbestanddeel glyfosaat is). Gezien de aard van de gerapporteerde effecten in een tweejarige rattenstudie met deze producten hebben verschillende voedselveiligheidsautoriteiten in Europa een evaluatie van de studie voorgenomen. De Europese Commissie heeft op 26 september 2012 het Europese Voedselveiligheidsagentschap EFSA (European Food Safety Authority) verzocht om een EFSA-oordeel van deze studie. Tevens heeft de Europese Commissie aan de EFSA gevraagd of de studie nieuwe wetenschappelijke inzichten bevat die aanleiding geven om de eerdere EFSA-beoordeling inzake GM-maïs (NK603) te hierzien. De EFSA heeft al op 19 september 2012 alle lidstaten van de EU benaderd en een wetenschappelijke risicobeoordeling van de studie aangekondigd gericht op de mogelijke consequenties van de uitkomsten van de studie voor de voedselveiligheid van genetisch gemodificeerde gewassen en de herbiciden op basis van glyfosaat.
In de publicatie[1] schrijven de auteurs dat een levenslange blootstelling aan een Roundup-tolerante genetisch gemodificeerde (GM) maïs en het

[1] Séralini, G-E., 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. Food and Chemical Toxicology. http://dx.doi.org/10.1016/j.fct.2012.08.005

onkruidbestrijdingsmiddel Roundup ernstige en letale ziekteverschijnselen veroorzaakt in de rat. De genetische modificatie zorgt ervoor dat de maïs bestand is tegen behandeling met de herbicide Roundup en is toegelaten voor import in de Europese Unie (Verordening 258/97). De mogelijke belangrijke risico's voor de volks- en diergezondheid waren voor de Ministeries van EL&I en VWS en het bureau Risicobeoordeling en onderzoeksprogrammering (BuRO) van de Nederlandse Voedsel- en Warenautoriteit (NVWA) reden om op de korte termijn een oordeel te vormen over de wetenschappelijke kwaliteit van het Franse onderzoek. Dit oordeel is vooruitlopend op en ondersteunend aan het oordeel van EFSA. De nadruk van de wetenschappelijke risicobeoordeling door NVWA-BuRO ligt op de validiteit van de conclusies en de eventuele consequenties voor de gezondheid van mens en dier.

Ondernomen acties

Direct na het openbaar maken van het artikel van Séralini en coauteurs heeft NVWA-BuRO op basis van de toen beschikbare informatie een eerste voorlopige inschatting gemaakt van de wetenschappelijke kwaliteit van het artikel en de eventuele gevolgen voor de huidige veiligheidsbeoordeling van genetisch gemodificeerde voedsel- en voedergewassen en herbiciden met de werkzame stof glyfosaat.

Op 24 september 2012 hebben de Ministeries van EL&I en VWS en NVWA-BuRO een wetenschappelijke beoordeling van het artikel gevraagd aan het frontoffice Voedselveiligheid van RIVM en RIKILT op basis van het eerste voorlopige oordeel van NVWA-BuRO. Hiertoe zijn een aantal verdiepende vragen voorgelegd. Op 27 september is van het frontoffice een eerste conceptantwoord ontvangen, en een definitief antwoord is op 1 oktober ontvangen.

Op 26 september 2012 heeft de directeur van NVWA-BuRO zijn eerste wetenschappelijke risicobeoordeling van de kwaliteit van het artikel van Séralini en coauteurs besproken in de vergadering van het EFSA Adviesforum in Parma.
Voor gedachtenwisseling en afstemming heeft NVWA-BuRO op 28 september 2012 deelgenomen aan de teleconferentie tussen vertegenwoordigers van EFSA en Europese lidstaten. In het bijzonder in Duitsland, Frankrijk en België zijn ook (voorlopige) risicobeoordelingen van de studie van Séralini en coauteurs uitgevoerd. Duitsland heeft getracht nadere informatie van de onderzoekers te verkrijgen (ruwe data, logboeken). De onderzoeksgroep van Séralini heeft echter niet gereageerd op dit verzoek. Frankrijk heeft aan de leverancier van de geteste ratten, Harlan, informatie over de rattensoort opgevraagd. Op 1 oktober 2012 is deze laatste informatie van het HCB (Haut Conseil des Biotechnologies) uit Frankrijk ontvangen.

De wetenschappelijke risicobeoordeling van RIVM en RIKILT en de informatie-uitwisseling met Europese lidstaten vormde de basis voor het definitieve advies van de directeur BuRO van de NVWA.

De evaluatie

NVWA-BuRO heeft aan het frontoffice RIVM-RIKILT Voedselveiligheid gevraagd de wetenschappelijke kwaliteit van het artikel te beoordelen. Hierbij gaat het ondermeer om proefopzet, uitvoering, interpretatie van resultaten, kwaliteit van de gebruikte proefdieren en statistiek. Ook is gevraagd een inschatting te maken van mogelijke consequenties voor de huidige methodiek van beoordeling van genetisch gemodificeerde gewassen en glyfosaathoudende gewasbeschermingsmiddelen om de gezondheidsrisico's voor mens en dier te kunnen schatten, waaronder de toepassing van de zogenoemde 90-dagen voederstudie. RIVM en RIKILT hebben tevens de eerste conclusies van de BuRO-risicobeoordeling van de Franse studie onderworpen aan een review (bijlage 1: Front Office beoordeling studie Séralini et al_definitief).
Daarnaast heeft NVWA-BuRO de beschikbare wetenschappelijke literatuur verkend over het testen van mogelijke schadelijke effecten voor mens en dier van de blootstelling aan GM-gewassen en het reguliere carcinogenteitsonderzoek in het algemeen.

Conclusies

Naar aanleiding van de wetenschappelijke risicobeoordeling van de studie van Séralini en coauteurs (2012) concludeert NVWA-BuRO dat de Franse onderzoekers verbanden leggen tussen behandeling en effecten die niet wetenschappelijk onderbouwd zijn.

In het bijzonder geldt het volgende.
- Door de gebruikte rattensoort in combinatie met de te kleine onderzoekspopulatie, geen vergelijking met een actuele of eigen databank van historische controlegroepgegevens (HCD) van de rattensoort en het ontbreken van een statistische analyse is er een grote kans dat de Franse onderzoekers een verkeerd beeld van de resultaten van de tweejarige voederproef in de rat hebben gegeven. Door de grotere kans op spontane kankers en tumoren en overige gezondheidsproblemen na twee jaar blootstelling kunnen de waargenomen verschillen tussen controle en behandelde groepen berusten op toeval, een risico dat toeneemt als de onderzoeksgroep niet groter is dan tien proefdieren (bijlage 1);
- De verhouding GM-maïs (NK603)/standaard knaagdiervoeder was niet gelijk in alle behandelde diergroepen. Het kan ook betekenen dat de gezondheidseffecten werden veroorzaakt door deze verschillen in de diëten en niet door de GM-maïs (NK603) of het herbicide glyfosaat.

- De fracties dieren met kankers nemen niet duidelijk toe met toenemende doses van GM-maïs (NK603) of glyfosaat. De auteurs geven aan dat sprake is van een 'threshold response' opgewekt door een invloed op de hormoonhuishouding. Dit is wetenschappelijk geen zinnige conclusie, omdat drempels met 10 dieren per dosisgroep volledig buiten het statistisch waarneembare gebied liggen.
- Van de stof glyfosaat, de actieve component in Roundup, of metabolieten ervan in gewassen, is niet eerder aangetoond dat deze kankerverwekkend is.
- Het onderzoek is qua opzet, uitvoering en rapportage ongeschikt om een uitspraak te kunnen doen over de veronderstelde niet-dosisgerelateerde hormoonontregeling door blootstelling aan de GM-maïs (NK603) of de stof glyfosaat. Een dergelijke conclusie vraagt om veel meer proefdieren en een goede statistische analyse.
- De biochemische veranderingen tussen behandelde groepen en controlegroep zijn niet te verifiëren: onderliggende data zijn niet gepubliceerd en de statistische methode (two class discriminant analyse) voor de data-analyse is erop gericht verschillen te vinden in plaats van te onderzoeken of er verschillen tussen onderzoeksgroep en controlegroep aangetoond kunnen worden.

Uit de nabeschouwing van de teleconferentie met EFSA en de lidstaten België, Duitsland en Frankrijk wordt geconcludeerd dat de conclusies van hun voorlopige risicobeoordelingen geheel in lijn zijn met de Nederlandse bevindingen (bijlage 2: TC minutes 28092012_1stMTG final).

Advies NVWA-BuRO

Gelet op de slechte wetenschappelijke kwaliteit van het artikel, zoals beoordeeld wordt door BuRO, het frontoffice RIVM-RIKILT, BfR in Duitsland, ANSES en HCB in Frankrijk, EFSA in Italië en het WIV-ISP in België adviseer ik om de huidige methodiek van de voedsel- en voederveiligheidsbeoordeling van GM-gewassen (guidance EFSA[a,b,c], 2011) en de herbicide glyfosaat (EFSA[a,b], 2009) niet te wijzigen. Verder adviseer ik u het oordeel van EFSA af te wachten die naar verwachting in oktober 2012 nog zal verschijnen.

Voor de verdere uitleg en onderbouwing van mijn wetenschappelijke risicobeoordeling van de studie verwijs ik naar deel 2 van mijn advies met de bijlagen 1 en 2.

Wat er verder nog gaat gebeuren

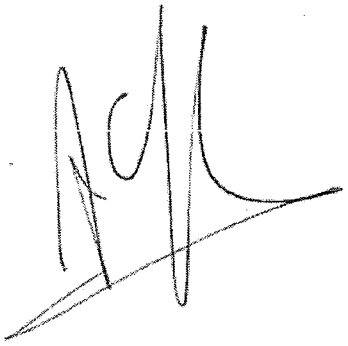
EFSA heeft aangekondigd begin oktober 2012 een eerste concept EFSA-oordeel naar buiten te brengen.
EFSA heeft aangekondigd eind oktober 2012 een definitief EFSA-oordeel van het artikel van Séralini en coauteurs naar buiten te brengen.

Bureau Risicobeoordeling &
onderzoeksprogrammering

Datum
1 oktober 2012

BuRO, zal evenals BfR uit Duitsland en ANSES uit Frankrijk, in oktober 2012 proberen nadere informatie van de onderzoekers te verkrijgen waarbij het gaat om ruwe gegevens van de studie, studieprotocollen en logboeken, qPCR-analyse van DNA monsters van GM-maïs (NK603), chemische samenstelling van de formuleringen GT Plus (glyfosaat) en WeatherMAX (glyfosaat), histopathologische gegevens van de individuele proefdieren, en chemische analyses van onder andere het diervoeder. Collega's van HCB uit Frankrijk hebben toegezegd begin oktober de informatie van de historische controlegegevens van de rattensoort (Harlan Sprague-Dawley) aan BuRO door te sturen. Op 1 oktober 2012 zijn deze gegevens ontvangen.

Mocht er aanleiding voor zijn, dan komt BuRO met een vervolgadvies over de wetenschappelijke risicobeoordeling van de studie van Séralini en coauteurs.



Bureau Risicobeoordeling &
onderzoeksprogrammering

Datum
1 oktober 2012

Deel 2

Onderbouwing van de beoordeling van de publicatie van Séralini en coauteurs over gezondheidsrisico's voor mens en dier na vervoederen van Roundup-tolerante GM-maïs en Roundup herbicide aan ratten

Achtergrond

Op 19 september 2012 publiceerde de EFSA (Europese Voedselautoriteit) een persbericht dat de Autoriteit de voorpublicatie van Séralini en medeauteurs in het wetenschappelijk tijdschrift Food and Chemical Toxicology met spoed zou bestuderen op consequenties voor de voedselveiligheid van genetisch gemodificeerde gewassen en glyfosaat. In de publicatie (Séralini et al. 2012) met als titel "Long term toxicity of a Roundup herbicide and Roundup-tolerant genetically modified maize" stellen Franse onderzoekers dat de dagelijkse blootstelling aan een Roundup-tolerante genetisch gemodificeerde (GM) maïs en het onkruidbestrijdingsmiddel Roundup na twee jaar ernstige en letale ziekteverschijnselen veroorzaakt in de rat. De genetische modificatie, overexpressie van het EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) transgen in NK603, zorgt ervoor dat de GM-maïs (NK603) bestand is tegen behandeling met de herbicide Roundup (de actieve stof is glyfosaat), dat is toegelaten voor import in de Europese Unie (Verordening EC 258/97 en Verordening EC 1829/2003[2]). De voorpublicatie heeft wereldwijd media-aandacht gekregen en ophef veroorzaakt met betrekking tot het onderzoek voor de bepaling van de voedselveiligheid van genetisch gemodificeerde gewassen en het testen van glyfosaatformuleringen op veiligheid voor mens, dier en milieu. Vooral het toepassen van de zogenoemde 90-dagen studie met het hele GM-gewas wordt in het artikel ernstig bekritiseerd (zie ook Séralini et al. 2011). Voor bureau Risicobeoordeling en onderzoeksprogrammering (BuRO) van de Nederlandse Voedsel- en Warenautoriteit (NVWA) was het persbericht van EFSA daarom aanleiding voor een risicobeoordeling van de wetenschappelijke kwaliteit van de Franse studie.

Vragen die gesteld zijn

Wat is het wetenschappelijk oordeel van het frontoffice RIVM-RIKILT Voedselveiligheid over het onderzoek van Séralini en coauteurs (2012)?

Daarbij zijn volgende subvragen van belang:

1. Zijn de argumenten van Séralini et al. om géén proefprotocol met een groepsgrootte van n=50 te gebruiken wetenschappelijk valide?

[2] EFSA Journal (2009) 1137, 1-50.

Citaat: "we had no reason to settle at first for a carcinogenesis protocol using 50 rats per group. However we have prolonged the biochemical and hematological measurements or disease status recommended in combined chronic studies using 10 rats per group (up to 12 months in OECD 453). This remains the highest number of rats regularly measured in a standard GMO diet study."

2. Vindt het frontoffice RIVM-RIKILT Voedselveiligheid de kritiek van Séralini et al. met betrekking tot de geadviseerde 90-dagen voederproef terecht en onderbouwd?

3. Is de stof glyfosaat en/of residuen kankerverwekkend?

4. Wat vindt het frontoffice RIVM-RIKILT Voedselveiligheid van de kwaliteit van de proefopzet en het rapporteren van resultaten, en de statistische analyse van eindpunten in relatie tot een gangbare toxicologische werkwijze?

5. Wat is het oordeel van RIVM en RIKILT over de verklaringen die onderzoekers geven ten aanzien van de oorzaak-effect relaties, zoals enerzijds een niet-lineaire hormoonontregeling door glyfosaat of anderzijds een niet-lineair effect door het transgen of metabole verstoringen in de maïs als gevolg van de insertie?

Opzet

NVWA-BuRO heeft aan het frontoffice RIVM-RIKILT Voedselveiligheid gevraagd de wetenschappelijke kwaliteit van het artikel van Séralini et al. (2012) te beoordelen en conclusies van de Franse onderzoekers nader te verifiëren. Daarnaast is door NVWA-BuRO de beschikbare wetenschappelijke literatuur verkend over het testen van mogelijke schadelijke effecten voor mens en dier van de blootstelling aan GM-gewassen. De gebruikelijke toxicologische werkwijze met betrekking tot carcinogeniteitsonderzoek zoals is voorgeschreven door diverse autoriteiten (o.a. OECD, EPA, NTP, EMA, ECHA) is ook nader bestudeerd.

Ook heeft NVWA-BuRO deelgenomen aan een Europa-brede uitwisseling van voorlopige conclusies van kritische oordelen van de verschenen studie van Séralini et al. (2012), en zijn de mogelijke consequenties voor de toelating van GM-maïs (NK603) en Roundup onderzocht. Daartoe heeft de EFSA een teleconferentie georganiseerd met lidstaten die al actief bezig zijn met deze kwestie (bijlage 2).

RIVM en RIKILT hebben vervolgens de eerste conclusies van het BuRO-onderzoek en een aantal verdiepende vragen onderworpen aan een risicobeoordeling (bijlage 1).

Op basis van de wetenschappelijke risicobeoordeling door het frontoffice RIVM-RIKILT Voedselveiligheid en de consultatie van EFSA en lidstaten is het advies van NVWA-BuRO afgerond.

De onderzoeksresultaten

De studie van Séralini en medeauteurs (2012) is gecompliceerd van opzet en volgt niet éénduidig de OECD richtlijnen voor proefdieronderzoek. In het algemeen is de studie onvolledig gepubliceerd. Er ontbreken in het artikel een veelvoud aan essentiële gegevens nodig voor een beoordeling van de resultaten en de op basis ervan getrokken conclusies (bijlagen 1 en 2). Zo is voor de teelt/veldproef van de GM-maïs (NK603) de glyfosaatformulering WeatherMAX (540 g/L glyfosaat) en voor de dierproef het middel GT Plus (450 g/L glyfosaat) gebruikt. Terecht stellen de auteurs vast dat dergelijke formuleringen adjuvants of overige actieve ingrediënten kunnen bevatten die niet zijn getoetst onder condities van een levenslange blootstelling aan proefdieren. De Franse onderzoekers verzuimen details over samenstelling te geven of aan te tonen dat beide formuleringen equivalent zijn.

Overige essentiële details die nodig zijn voor een goede risicobeoordeling van de Franse studie ontbreken, zoals bijvoorbeeld gegevens over analyseresultaten van de GM-maïs (NK603) en de controle maïs, de rattenvoeders, de voeder- en waterconsumptie, en over de groei en lichaamsgewichten van de ratten, en of de studie al dan niet geblindeerd was. Een statistische analyse van bijvoorbeeld consumptie, groei, mortaliteit, en kankerincidentie en multipliciteit is niet uitgevoerd. En data over de uitgevoerde statistische analyse van de biochemische parameters ontbraken.

EFSA adviseert om een 90-dagen studie met het hele GM-gewas alleen dan uit te voeren indien daar door toxicologisch vooronderzoek aanleiding voor is (EFSA[a,b,c], 2011). Dit is niet het geval indien een genetisch gemodificeerd gewas 'substantieel equivalent' door bijvoorbeeld het GMO-panel van EFSA is bevonden aan de niet-GM isogene counterpart (EFSA, 2011). In het artikel uiten Séralini en medeonderzoekers kritiek op deze EFSA 'guidance' voor onderzoek naar de veiligheid van een genetisch gemodificeerd voedsel- en/of voedergewas (zie ook Séralini et al. 2011). Correct merken de auteurs op dat dierproeven tot nu toe niet worden vereist voor de vaststelling van 'substantieel equivalente' GMOs, Séralini et al. vinden dit echter een fout in het huidige veiligheidsonderzoek. Zij verwerpen principieel het door de EFSA geadviseerde gangbare gebruik van een voederproef die 90-dagen duurt indien daar door toxicologisch vooronderzoek aanleiding toe is. Het is de visie van Séralini et al. (2011, 2012) dat een blootstelling gedurende drie maanden onvoldoende is om

effecten voor de volks- en diergezondheid te kunnen beoordelen. In tegenstelling tot de 'guidance' van het GMO panel (EFSA[a,b,c], 2011) zijn Séralini en coauteurs van mening dat gezondheidseffecten van GM-gewassen onderzocht moeten worden met behulp van chronische studies; bijvoorbeeld overeenkomend met de levensduur van het proefdier.

De onderzoeksgroep van Gilles-Eric Séralini van de Universiteit van Caen in samenwerking met de Parijse commissie CRIIGEN heeft daarom een tweejarige voederstudie met ratten uitgevoerd waarbij zij gebruik heeft gemaakt van de genetisch gemodificeerde NK603 Roundup Ready-maïs van Monsanto (VS) en het onkruidbestrijdingsmiddel glyfosaat (Roundup). Hun studieopzet komt er in het kort op neer dat de onderzoekers een chronisch onderzoek in de rat uitvoerden met de Harlan Sprague-Dawley rat en fysiologische en biochemische bepalingen uitvoeren zoals gebruikelijk is voor een 90-dagen voederstudie de effecten in het proefdier bestudeerden. Één controlegroep, die gewone maïs at (33% in standaard proefdiervoer) en schoon drinkwater, werd vergeleken met zes groepen die het gengewas (NK603) geteeld met of zonder Roundup in het voer (11%, 22% en 33%) kregen of met drie groepen die gewone maïs aten (33% in standaard proefdiervoer) en het middel met merknaam GT Plus in het drinkwater (50 ng/L, 400 mg/kg en 2,25 g/L glyfosaat). Zoals hierboven is beschreven is de samenstelling van de gebruikte glyfosaat-formuleringen niet gegeven wat wel zou moeten, omdat het niet ondenkbaar is dat een langdurige blootstelling aan de overige (hulp-)stoffen in de glyfosaat-formulering GT Plus negatieve effecten op de gezondheid van het proefdier zouden kunnen induceren (persoonlijke mededeling van Dr. Lars Niemann, BfR uit Duitsland). Het is merkwaardig dat auteurs de dosering glyfosaat van de middelste testgroep uitdrukken in mg per kilogram lichaamsgewicht en niet vermelden voor de overige twee testgroepen.

Controlegroepen die respectievelijk zijn gevoederd met 11 en 22% gewone maïs ontbreken in het proefprotocol. Ook ontbreken er resultaten van de uitgevoerde qPCR analyse van DNA monsters van de GM-maïs (NK603), auteurs refereerden dergelijke testen wel te hebben uitgevoerd.

Dit heeft tot gevolg dat de ratio maïs/standaard dieet niet in alle groepen gelijk blijkt te zijn geweest. Eventuele effecten zouden dus veroorzaakt kunnen zijn door onder andere verschillen in samenstelling van het dieet. Bijvoorbeeld het is bekend dat de voedselopname een invloed heeft op de tumorgroei (Tucker, 1979).

Vastgesteld wordt dat essentiële details die nodig zijn voor een goede risicobeoordeling van de inhoud en het onderzoek in het artikel van Séralini et al. ontbreken.

De auteurs hebben waargenomen dat de NK603gevoerde ratten eerder stierven met een versterkend effect op sterfte (mortaliteit) indien het gengewas met Roundup (WeatherMAX?) was behandeld. Ook duiden hun resultaten erop dat de genetisch gemodificeerde maïs met resistentie tegen glyfosaat tot vijfmaal meer borstkliertumoren (vrouwtjesratten) en viermaal meer palpeerbare nier- en/of huidtumoren (mannetjes) induceerde bij levenslange blootstelling. Het aantal neoplasieën en grootte per individuele rat zijn niet gepubliceerd. De auteurs vermelden slechts de relatieve percentages of ratio's van neoplasieën (goed- en kwaadaardige kankers) per groep. In de publicatie van Séralini en coauteurs ontbreekt de histopathologische typering van neoplasieën per proefdier zoals gebruikelijk is in toxicologische publicaties.

Om resultaten van een carcinogineitstudie accuraat te kunnen beoordelen moet rekening worden gehouden met de incidentie van spontaan voorkomende tumortypen bij de Sprague-Dawley (SD) rat, aangezien het percentage melkkliertumoren bijvoorbeeld kan oplopen tot 50% in controlegroepen (Mann et al., 1996; Nakazawa et al. 2001) of hypofysetumoren (adenomas) tot 49% (mannetjes) en 75% (vrouwtjes) volgens Baldrick (2005), en zelfs na verloop van tijd kan variëren binnen een laboratorium (genetische drift).

Het is een goede toxicologische werkwijze om met behulp van een databank met historische controledata (HCD) van de Harlan SD-rat te onderzoeken of de tumorresponsen in de studie door bijvoorbeeld genetische drift ongebruikelijk zou kunnen zijn door te vergelijken met wat normaal is. Dit doet men door vergelijking met het aantal en type neoplasieën in controledieren van een grote serie eerder uitgevoerde studies (bijv. Baldrick, 2005; Elmore en Peddada, 2009). Ook Séralini et al. pasten dit principe toe door hun effecten in behandelde groepen te vergelijken met gepubliceerde HCD's van spontane incidenties in de gebruikte rattensoort (Chandra et al. 1992; Brix et al. 2005). Zowel het National Toxicology Program (NTP) van het departement Health and Human Services (NIEHS) uit de VS als het Europese Agentschap voor Medicijnen (EMA) adviseren echter het gebruik van HCD's die studies samenvatten van de laatste 7 jaar, respectievelijk de laatste 5 jaar. Dit is door Séralini en coauteurs niet gedaan. Daarom zouden de Franse onderzoekers alsnog hun eigen HCD van de SD-rat of die van Harlan uit Frankrijk moeten overleggen voor nader onderzoek op biologische significantie van waargenomen effecten.

Niet onvermeld mag blijven dat een voedingskundige van de leverancier Harlan uit Amerika recent in een interview met Tim Worstall in Forbes aangeeft dat Harlan geen onderscheid maakt tussen GM-maïs en niet-genetische gemodificeerde varianten wat betreft de samenstelling van proefdiervoeders (Worstall, 2012). Met andere woorden het is goed denkbaar dat in Amerika herbicide-tolerante GM-maïs al langer aan ratten is gevoederd zonder het waarnemen van nadelige effecten op de diergezondheid.

Conclusies: antwoord op vragen

1. Zijn de argumenten van Séralini et al. om géén proefprotocol met een groepsgrootte van n=50 te gebruiken wetenschappelijk valide?

Nee, het aantal proefdieren van 10/sexe/dosering is te laag om een uitspraak te kunnen doen over verschillen in tumorincidenties tussen de diverse onderzoeksgroepen, d.w.z. ratten met of zonder kanker. Voor een chronische toxiciteitstudie zou het aantal proefdieren voldoende zijn (zie OECD Richtlijn 453), mits ondersteund met gegevens van groepen van 50 dieren/sexe/dosering in de gecombineerde carcinogeniteitstudie. Daarbij hebben Franse onderzoekers een rattensoort (Sprague-Dawley) gebruikt die bekend staat gevoelig te zijn voor de 'spontane' ontwikkeling – hogere incidentie - van melkklierkanker en hypofysetumoren en een grotere kans op allerlei gezondheidsproblemen aan het eind van hun leven (na twee jaar blootstelling). Séralini en coauteurs hebben geen gegevens gepubliceerd van een actuele databank met historische controlegegevens (HCD) van de gebruikte rattensoort. Verder is van belang dat de Franse onderzoekers geen statistische analyse van de effecten van de verschillende behandelingen op mortaliteit en tumorincidentie hebben uitgevoerd.

2. *Vindt RIVM-RIKILT de kritiek van Séralini et al. met betrekking tot de geadviseerde 90-dagen voederproef terecht en onderbouwd?*

Nee, de kritiek van Séralini en coauteurs is niet goed onderbouwd. De onderzoekers zijn selectief in het citeren van publicaties waarin alleen mogelijke nadelige effecten van GMO's in proefdieren of van glyfosaatformuleringen in celkweek worden gerapporteerd zonder daarbij te vermelden dan meer dan 100 publicaties geen nadelige effecten rapporteren.

3. *Is de stof glyfosaat en/of residuen kankerverwekkend?*

Nee, de stof glyfosaat is in 2000 (EU) en 2004 (JMPR) beoordeeld en uitgevoerde carcinogeniteitstudies in muis en rat tonen aan dat glyfosaat geen carcinogeen is. De voornaamste metaboliet van glyfosaat in plant en milieu vertoont een lagere toxiciteit.

4. *Wat vindt het RIVM-RIKILT van de kwaliteit van de proefopzet en het rapporteren van resultaten, en de statistische analyse van eindpunten in relatie tot een gangbare toxicologische werkwijze?*

De kritiek van NVWA-BuRO wordt gedeeld (bijlage 1), waarbij RIVM en RIKILT opmerken dat Séralini en coauteurs geen statistische evaluatie hebben uitgevoerd met betrekking tot de waargenomen mortaliteit en tumorincidentie. Indien auteurs de gebruikelijke statistische analyse door

paarsgewijs toetsen van doseringsgroepen met controle(s) (en met een actuele HCD van de SD-rat) zouden hebben uitgevoerd is de verwachting dat er geen significante verschillen zijn in de resultaten van mortaliteit en tumorincidentie tussen de diverse groepen.

5. *Wat is het oordeel van RIVM-RIKILT over de verklaringen die onderzoekers geven ten aanzien van de oorzaak-effect relaties, zoals enerzijds een niet-lineaire hormoonontregeling door glyfosaat of anderzijds een niet-lineair effect door het transgen en/of metabole verstoringen als gevolg van de insertie?*

De studie van Séralini is qua uitvoering en rapportage ongeschikt om een uitspraak te kunnen doen over de veronderstelde niet-dosisgerelateerde hormoonontregeling. Een dergelijke conclusie zou zeer veel dieren vergen en een goede statistische analyse. De auteurs leggen verbanden tussen behandeling en effecten (oorzaak en gevolg) die op grond van de resultaten niet wetenschappelijk onderbouwd zijn.

Het is bijvoorbeeld bekend dat spontane hypofysetumoren vaak voorkomen bij Sprague-Dawley ratten met borstklierfibroadenoma's. Percy and Barthold rapporteerden in 1993 dat 90% van de ratten met borstklierfibroadenoma's (circa 80-95% van alle borsttumoren) hypofysetumoren hebben. Dit suggereert een verband tussen beide maligniteiten onder invloed van verhoogde concentraties aan circulerende groeihormonen (Pecceu, 2010).

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Bureau Risicobeoordeling &
onderzoeksprogrammering

Datum
1 oktober 2012

www.forbes.com/.../proof-perfect-that-the-seralini-paper-on-gm-corn-and-cancer-in-rats-is-rubbish/

Bijlage 1
NVWA/BuRO/2012/3569

Rijksinstituut voor Volksgezondheid
en Milieu
*Ministerie van Volksgezondheid,
Welzijn en Sport*

RIKILT
WAGENINGEN UR

RIVM-RIKILT FRONT OFFICE VOEDSELVEILIGHEID

BEOORDELING INZAKE ARTIKEL SÉRALINI et al. 2012 in FOOD and CHEMICAL TOXICOLOGY

Risicobeoordeling aangevraagd door:	Hub Noteborn (NVWA, BuRO)
Risicobeoordeling opgesteld door:	*RIVM en RIKILT*
Datum aanvraag:	24-09-2012
Datum risicobeoordeling:	01-10-2012 (definitieve versie)
Coördinator:	Suzanne Jeurissen
Opstellers risicobeoordeling:	Gerrit Wolterink, Wout Slob, Karin Mahieu (RIVM), Maryvon Noordam (RIKILT)
Toetsers risicobeoordeling:	Bernadette Ossendorp, Mirjam Luijten (RIVM), Boet Glandorf (BGGO/RIVM), Esther Kok (RIKILT)
Projectnummers:	V/320110/12/AA en V/320800/12/AA

Onderwerp

In het wetenschappelijke tijdschrift Food and Chemical Toxicology is een studie gepubliceerd, geschreven door Séralini G-E, Clair E. et al. (2012) met als titel: "Long term toxicity of a RoundUp herbicide and RoundUp-tolerant genetically modified maize". In de publicatie wordt gesteld dat nu is aangetoond dat levenslange blootstelling aan een glyfosaat-tolerante GM-maïs (NK603) en/of een commerciële glyfosaat-bevattende formulering (RoundUp) leidt tot zeer ernstige ziekteverschijnselen in de Sprague Dawley rat. De publicatie heeft wereldwijd tot ophef geleid en binnen de Europese Unie gaan stemmen op om de grenzen te sluiten voor genetisch gemodificeerde gewassen.

Vraagstelling

Wat is het wetenschappelijk oordeel van RIVM-RIKILT over het onderzoek van Séralini en medeauteurs (2012)?

Daarbij zijn de volgende subvragen van belang:

1. Zijn de argumenten van Séralini et al. om géén proefprotocol met een groepsgrootte van n=50 te gebruiken wetenschappelijk valide?

Citaat: "we had no reason to settle at first for a carcinogenesis protocol using 50 rats per group. However we have prolonged the biochemical and hematological measurements or disease status recommended in combined chronic studies using 10 rats per group (up to 12 months in OECD 453). This remains the highest number of rats regularly measured in a standard GMO diet study."

2. Vindt RIVM-RIKILT de kritiek van Séralini et al. met betrekking tot de geadviseerde 90-dagen voederproef terecht en onderbouwd?

Zie bijvoorbeeld publicatie van o.a.: Snell et al. Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: A literature review. Food and Chemical Toxicology, Volume 50, Issues 3–4, March–April 2012, Pages 1134-1148 (hieronder) en de EFSA-opinie "Safety and Nutritional Assessment of GM Plant derived Foods/Feed. The role of animal feeding trials"(2007) (bijlage)

3. Is de stof glyfosaat en/of residuen kankerverwekkend?

4. Wat vindt het RIVM-RIKILT van de kwaliteit van de proefopzet en het rapporteren van resultaten, en de statistische analyse van eindpunten in relatie tot een gangbare toxicologische werkwijze?

5. Wat is het oordeel van RIVM-RIKILT over de verklaringen die onderzoekers geven ten aanzien van de oorzaak-effect relaties, zoals enerzijds een niet-lineaire hormoonontregeling door glyfosaat of anderzijds een non-lineair effect door het transgen en/of metabole verstoringen als gevolg van de insertie?

Conclusie

1) Om uitspraken te kunnen doen over het kankerverwekkend zijn van een stof, product of GGO, is de door Séralini gebruikte opzet niet geschikt. Daarvoor had een hoger aantal ratten per groep ingezet moeten worden. Vanwege de kleine groepsgrootte kan toeval de gevonden verschillen tussen de controle en de behandelde groepen in deze studie verklaren. Een verdere tekortkoming is dat in de publicatie geen statistische analyse van de aantallen waargenomen dieren met tumoren (en enkele andere effecten) is opgenomen.
2) In de publicatie van Séralini et al. (2012) wordt de EFSA niet genoemd, wel staat vermeld dat 'Currently, no regulatory authority requests mandatory chronic animal feeding trials to be performed for edible GMOs and formulated pesticides'. EFSA adviseert om een 90-dagen studie met het hele GGO-product alleen uit te voeren indien daar aanleiding voor is. Dit is niet het geval indien een GGO-product 'substantieel equivalent' is bevonden aan de niet-GGO isogene counterpart (EFSA, 2011). De opmerking van Séralini et al. dat dierproeven tot nu toe niet worden vereist voor substantieel equivalente GGOs is dus correct, maar er is geen aanleiding om dit te veranderen.
3) Volgens de JMPR (2004) en de EC (2002) zijn glyfosaat en/of residuen van glyfosaat niet kankerverwekkend.
4) Door het lage aantal dieren per groep is het zeer waarschijnlijk dat waargenomen verschillen berusten op toeval. De fracties dieren met dergelijke tumoren nemen niet duidelijk toe met toenemende dosis.
5) Deze studie is qua opzet, uitvoering en rapportage ongeschikt om een uitspraak te kunnen doen over de veronderstelde niet-dosisgerelateerde hormoonontregeling. Een dergelijke conclusie zou zeer veel dieren vergen en een goede statistische analyse. Een andere conclusie betreft de biochemische veranderingen in de dieren in de toxiciteitsproef. Deze conclusie is niet te verifiëren omdat de onderliggende data niet terug te vinden zijn in de publicatie. De auteurs leggen verbanden tussen behandeling en effecten die op grond van de resultaten niet wetenschappelijk onderbouwd zijn.

Toelichting

Vraag 1
Het aantal proefdieren 10/geslacht/dosis is te laag om een uitspraak te kunnen doen over het al dan niet kankerverwekkend zijn van een agens. Voor een chronische toxiciteitstudie is dit aantal voldoende (OECD 453), mits ondersteund met gegevens van de 50 dieren/geslacht/dosis in een gecombineerde carcinogeniteitsstudie. "For a thorough biological and statistical evaluation of the study each dose group should at least contain 50 animals of each sex. Each dose group and concurrent control group intended for the chronic phase of this study (OECD 453 and not TG 452, which requires a higher number) should contain at least 10 animals/sex." Conclusie: 10 dieren/geslacht/dosis is niet genoeg om uitspraken te kunnen doen over verschillen in aantallen dieren met of zonder kanker.
Hierbij kan worden opgemerkt dat in de publicatie van Séralini geen statistische analyse van de effecten van de verschillende behandelingen op de mortaliteit en tumorincidentie is uitgevoerd. De conclusies van Séralini t.a.v. tumoren en de andere gerapporteerde effecten hebben daarom geen enkele basis.

Vraag 2
De kritiek die Séralini heeft op de studie van Snell et al., 2011 is niet goed gefundeerd. De auteur haalt selectief enige publicaties aan waarin wel effecten van GGOs op proefdieren c.q. RoundUp formuleringen op celcultures worden gevonden, maar gaat echter geheel voorbij aan de vele (geschat meer dan 100) publicaties waarin GGOs gedurende 90 dagen of langer gevoerd zijn aan proefdieren en waarin geen effecten werden gevonden. Daarnaast wekt hij in dezelfde alinea de suggestie dat, als studies zouden zijn uitgevoerd volgens het protocol zoals beschreven in zijn eigen publicatie, chronische GMO voederstudies wel gezondheidseffecten zouden aantonen. Daar deze studie niet geschikt is om hierover enige uitspraak te doen is dit ook geen steekhoudend argument om de conclusies uit de studie van Snell et al. te ondermijnen.
In de publicatie van Séralini et al. (2012) wordt EFSA niet genoemd, wel meldt men dat 'Currently, no regulatory authority requests mandatory chronic animal feeding trials to be performed for edible GMOs and formulated pesticides'. EFSA adviseert om een 90-dagen studie alleen uit te voeren indien daar aanleiding voor is. Dit is niet het geval indien een GMO 'substantieel equivalent' is bevonden aan de niet-GGO isogene counterpart (EFSA, 2011). De opmerking van Séralini et al. dat dierproeven tot nu toe niet worden vereist voor substantieel equivalente GGOs is dus correct, maar er is geen aanleiding om dit te veranderen.

Vraag 3
Het herbicide met de commerciële naam RoundUp bevat als actieve stof glyfosaat. De overige bestanddelen van het gebruikte middel worden niet vermeld. Op basis van deze studie kan niet worden geconcludeerd dat (residuen van) glyfosaat (in de vorm van RoundUp,) of het genetisch gemodificeerde maïs alleen, of het genetische gemodificeerde maïs in combinatie met RoundUp of RoundUp alleen carcinogeen is (zie antwoorden op vragen 1 en 5 ten aanzien van de kwaliteit van de studie).

De toxiciteit van de actieve stof glyfosaat is in 2000 in de EU beoordeeld (EU, 2000) en in 2004 door JMPR beoordeeld (JMPR, 2004). De conclusie in beide evaluaties is dat glyfosaat niet carcinogeen is in goed uitgevoerde carcinogeniteit studies in muizen en ratten. Daarnaast concludeert de JMPR in 2011 dat AMPA (de voornaamste metaboliet in de plant en in het milieu) minder toxisch is dan glyfosaat (JMPR, 2011). De door de JMPR vastgestelde ADI voor glyfosaat en AMPA) van 1 mg/kg bw/day is gebaseerd op effecten op de speekselklieren gezien in de chronische/carcinogeniteit studies (NOAEL 100 mg/kg bw/day).

Opgemerkt wordt dat de auteurs aansturen op effecten ten gevolge van blootstelling via voeding/water aan een glyfosaat bevattend middel met de merknaam GTplus, door de auteurs aangeduid als RoundUp (wat anders is dan de actieve stof alleen) en via het eten van genetisch gemodificeerd NK603 maïs. Ze trekken dus niet direct de conclusies ten aanzien van de actieve stof glyfosaat zelf in twijfel.

Vraag 4
Séralini et al. hebben geen resultaten gerapporteerd van een statistische analyse op de effecten op mortaliteit en tumorincidentie. Indien deze wel zou zijn uitgevoerd dan zou de gebruikelijke analyse (paarsgewijs toetsen van dosis groepen met de controle) voor deze parameters geen statistisch significante verschillen aangetoond hebben. Dat valt op basis van een studie met deze kleine aantallen dier per dosis ook niet te verwachten. Door het geringe aantal dieren per groep is het zeer waarschijnlijk dat de waargenomen verschillen berusten op toeval.
De resultaten voor dieren met tumoren worden (in tabel 2) samengevat door de aantallen tumoren per dier bij elkaar op te tellen, terwijl het aantal dieren met een of meer tumoren tussen haakjes vermeld staat. In carcinogeniteit-studies wordt normaal gesproken het aantal dieren met tumoren vermeld en niet het totaal aantal tumoren.
Op de biochemische data is wel een statische analyse uitgevoerd. Echter, de onderliggende data zijn niet terug te vinden in de publicatie en de gekozen statistische methode (two class discriminant analyse) voor de data-analyse lijkt erop gericht verschillen te vinden in plaats van te onderzoeken of er verschillen in biochemische parameters tussen de onderzoeksgroepen en de controlegroep aangetoond kunnen worden. Deze conclusies betreffende de biochemische veranderingen in de dieren in de toxiciteitsproef zijn niet te verifieren.

De fracties dieren met tumoren nemen niet duidelijk toe met toenemende dosis. Séralini et al. voeren aan dat het ontbreken van een dosis-respons relatie te wijten is aan een effect op de hormoonhuishouding, echter zonder hiervoor verdere uitleg aan te dragen. Met het gehanteerde geringe aantal dieren is te verwachten dat de waargenomen fracties dieren met respons grillig zullen zijn, zelfs al zou er wel degelijk sprake van een normale dosis-respons relatie zijn.

Verder wordt het zeer onwaarschijnlijk geacht dat NK603 maïs dezelfde werking zou hebben als de in de studie gebruikte glyfosaat formulering.

Enige aanvullende punten van kritiek:

- Uit de materialen en methoden blijkt dat bij de controle dieren en bij de dieren die de hoogste dosis GGO maïs ontvingen, respectievelijk 33 % controle maïs of 33% GGO (NK603) maïs in het standaard dieet was verwerkt. De dieren die de lagere doses GGO maïs ontvingen hadden een standaard dieet waarin respectievelijk 11 en 22% GGO maïs was verwerkt. De controles met resp. 11 en 22% controle maïs ontbreken dus. De ratio maïs/standaard dieet was dus niet gelijk in alle groepen. Eventuele effecten kunnen dan ook veroorzaakt worden door verschillen in het dieet die niets met het GGO maïs te maken hebben.
- De studie is zeer gebrekkig gerapporteerd. Vele details die nodig zijn voor een goede beoordeling ontbreken, zoals bijvoorbeeld gegevens over analyseresultaten van de GGO maïs en de controle maïs, de rattenvoeders, voeder- en waterconsumptie, en over de groei van de ratten, en over het al dan niet geblindeerd zijn van de studie. Een statistische analyse van o.a. consumptie, groei, mortaliteit, en kankerincidentie en multipliciteit is niet uitgevoerd dan wel niet in het artikel opgenomen, en essentiële gegevens over de wel uitgevoerde statistische analyse van de biochemische data ontbreken.

- De auteurs claimen een effect op de hormoonhuishouding. In de omschrijving van de uitvoering van de studie (materiaal en methoden) lijkt er echter geen rekening gehouden te zijn met de fase van de cyclus van de vrouwelijke dieren op moment van bloedafname. Dit in combinatie met een laag aantal dieren per groep maakt dat de gevonden verschillen in estradiol spiegels zeer goed kunnen berusten op toeval.

Vraag 5
De studie van Séralini is qua uitvoering en rapportage ongeschikt om een uitspraak te kunnen doen over de veronderstelde niet-dosisgerelateerde hormoonontregeling. Een dergelijke conclusie zou zeer veel dieren vergen en een goede statistische analyse. De auteurs leggen verbanden tussen behandeling en effecten die op grond van de resultaten niet wetenschappelijk onderbouwd zijn.

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Afkortingen:

GM	=	Genetically Modified
GMO	=	Genetically Modified Organism
GGO	=	Genetische Gemodificeerd Organisme

BIJLAGE 2
NVWA/BuRO/2012/3569

BIJLAGE 2

European Food Safety Authority

Emerging Risks Unit **28 September 2012**

Teleconference with Member States on Séralini et al. study 28 September 2012, 10.30-12.30 1st Meeting Report

Participants: EFSA Response Team (RT)

- PB (GMO - Chair)
- TR (EMRISK – administrative coordinator)
- AC (EMRISK)
- AR (EMRISK – minute taker)
- DV (SAS)
- SB (SAS)
- HF (PRAS)
- MT (PRAS)
- EW (GMO)
- CO (GMO)
- KH (EDIT)
- SN (AFSCO)
- RH (EDIT)

Participants: Member States

- PC (WIV-ISP; BE)
- PH (WIV-ISP; BE)
- HN (NVWA; NL)
- JCL (Anses; FR)
- CG (HautConseilBiotechnologies, FR)
- AP (BfR; DE)
- LN (BfR; DE)
- AL (BfR; DE)

Apologies

- JF (EFSA, AFSCO)

1. Agenda

Time	Topic	Who
10:30-10:45	General introduction and tour de table	EFSA, PB
10:45-11:00	Mandate, approach and preliminary conclusions	EFSA, SB
11:00-11:15	Mandate, approach and preliminary conclusions	Netherlands, HN
11:15-11:30	Mandate, approach and preliminary conclusions	Germany, LN, AP & AL
11:30-11:45	Mandate, approach and preliminary conclusions	France, CG & JCL
11:45-12:00	Mandate, approach and preliminary conclusions	Belgium, PC & PH
12:00-12:15	Overview	EFSA and All
12:15-12:30	Identification of action points and follow up on exchange of information	All

2. Summary of key points

- **General introduction and tour de table**

PB welcomed MS and introduced rapidly the EFSA Response team (RT). No tour de table due to time constraint.

- **EFSA: Mandate, approach and preliminary conclusions**

Mandate: PB presented the mandate and agenda of the EFSA RT that was presented at the AF meeting on 26-27/09 at EFSA. EFSA needs to collaborate with MS in order to discuss scientific concerns and avoid divergence. EFSA is requested to review the paper and request clarifications from the authors as needed and to indicate if EFSA's opinion on NK603 and its stacks needs to be reconsidered. EFSA will issue a statement by mid-next week and a final assessment by the end of October. The outputs will be published on the EFSA website.

Approach: SB presented the approach of EFSA and the key structure and points of the statement :

- Abstract (aiming to be readable by general public)
- Scientific Approach:
    1. EU approach for toxicological assessment (GMO and Pesticides)
    2. Review of Séralini et al. paper (overview on the experiment and protocol; critical review with pending issues)
- Conclusions (aiming to be readable by general public)

Preliminary conclusions: The critical review addresses objectives, animals and feeding conditions, study design, sample size/power calculation, statistical methods, endpoint reporting. The pending issues highlight gaps and missing elements cited in the previous sections. In summary, it is concluded that this paper is of poor quality both in terms of reporting and clarity. The conclusions made by the authors cannot be supported and, based on the paper, there is no evidence for a need for re-evaluation of NK603 and glyphosate.

PB asked MS if they had any questions, clarification needs or any diverging views. France (JCL) asked who were the authors of the assessment. PB explained that the statement is drafted by EFSA staff from the SAS, GMO and PRAS Units.

- **Netherlands: Mandate, approach and preliminary conclusions**

Mandate: NVWA received a request from Secretary of the Ministry of Economic Affairs, Agriculture and Innovation (EL&I) and the Minister of the Ministry of Public Health, Welfare and Sports (VWS) to critically evaluate (review) the paper. Scientific advice should be delivered by 3th October.

Approach: multidisciplinary task force was set up by institutes RIKILT and RIVM, to analyse the paper by Seralini et al.

Preliminary conclusions: criticisms were raised to the study design (not compliant with OECD guidelines, rat strain Sprague Dawley not adequate due to high cancer incidence background, and no comparison with an own or other actual (i.e. covering studies of last 5 to 7 years) HCD of Harlan SD-rats), the sample size (low animal number in control and treatement groups), statistical analysis (basic and missing for many endpoints), and an overall lack of information (adjuvants/ingredients of WeatherMax and GT Plus, compositional analysis of GM maize and control maize, diets, feed and water consumption or growth rate etc.). To summarize, study design, way research was conducted and reporting are not suitable to be able to come to a science-based conclusion on, for instance, the supposed non-dose related endocrine disruption or non-dose related metabolic disturbance due to the insertion.

Additional information: Advice (opinion) will be published on the NVWA website by end of next week, and the Secretary of Agriculture (EL&I) will use NVWA's opinion to answer questions posed by members of the House of Representatives (Tweede Kamer) on October the 8th during the general consultation (AO) on "Pesticides".

- **Germany: Mandate, approach and preliminary conclusions**

Mandate: on behalf of the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) the Federal Institute for Risk Assessment (BfR) has received a request from the Department Genetic Engineering of the Federal Office of Consumer Protection and Food Safety (BVL) to evaluate the publication by Séralini et al., (2012). BfR is going to deliver an opinion on 28th September. Deadline for the final BVL report to the BMELV is 19th October. BfR has received a second request from the Department Plant Protection Products of the BVL asking for a scientific evaluation of the new data. An opinion should be delivered by 19th October.

Approach: the Seralini publication has been assessed by a multidisciplinary working group of experts from different BfR Departments. A letter with specific questions to the authors and asking for the complete study report was sent to Seralini 21th September . The BfR evaluation is confined to the study and does not address issues related to the risk assessment of GM plants as outlined in the respective EFSA guidance document.

Preliminary conclusions: BfR has noticed that for the first time a long-term feeding study was performed with a formulation containing the active ingredient glyphosate (Roundup), but no detailed information on the formulation was provided. The main criticisms and conclusions regarding the study are in line with those of the other MS, i.e.: not performed in line with relevant OECD Guidelines, low animal number per group in combination with a high rate of spontaneous tumours, information on diet composition and administered (glyphosate) doses missing, maize not analysed for mycotoxins, incomplete data reporting, no detailed specification of pathologies, practically no statistical analysis, anticipated endocrine effects not substantiated by experimental data. Therefore the main conclusions are not comprehensible. .

Additional information: the opinion is going to be published on the BfR website by the beginning of next week.

- **France: Mandate, approach and preliminary conclusions**

Mandate: ANSES and HCB received a joint mandate from the Ministries of Agriculture, Ecology, Health and Economy. The Mandate is asking HCB and Anses to analyse the study in order to determine whether it puts in question the conclusions of previous risk assessment of NK603, and in particular whether it can be considered as conclusive about the security of food and feed containing NK603. In addition, Anses is specifically asked to determine whether the study may put in question the conclusions of past Anses assessments of Roundup herbicide. Based on their analysis, HCB and Anses are asked to evaluate whether the protocol and the conclusions of the study put in question the current guidelines, or guidelines in preparation, with respect to sanitary risk assessment.

An opinion is requested by October 20th for the first two questions, and by November 20th for the last question,

Approach: Seralini publication will be assessed by multidisciplinary experts of just constituted working groups in Anses and in HCB.

Preliminary conclusions: no preliminary conclusions can be drawn at this stage as Anses/HCB expert WG have just started their expertise work; criticisms in line with other MSs.

Additional information: meetings with Industry and the authors have been planned. Moreover, control historical data have been sent by the animal supplier (Harlan).

- **Belgium: Mandate, approach and preliminary conclusions**

Mandate: For the GMO part, mandate received by the Biosafety Advisory Council (BAC) from the Minister of Public Health. Conclusions (critical review of the Seralini paper with the aim to evaluate whether the current decision for marketing of NK603 needs to be revised) are expected by the end of October. For the pesticide part, no official mandate but critical analysis of Toxicology Unit of WIV-ISP sent to the Competent Authority.

Approach: For the GMO part, multidisciplinary experts have been contacted. Their advices will be collected, and will serve as basis to draft the final advice of the BAC that will be sent to the Minister and published on the website of the BAC.

Preliminary conclusions: For the GMO part, it's too early to draw conclusions. For the pesticide part, comments were in agreement with the other MSs. In addition, lack of dose-response in the study and food composition analysis methodology have been highlighted.

- **All: Overview**

EFSA and MS share the same concerns about the publication of Séralini et al. on reporting and clarity, statistical analysis, sampling size, animal strain, etc. (see above).

In relation to the peer-review process leading to the publication of this study, Belgium (PC) suggested to write to the editor of the journal regarding the identified scientific weaknesses; This might be worthwhile, however, it should be noted that it seems that the scientific community is already reacting on this issue. It is understood that Elsevier has received a significant number of letters requesting the reconsideration of the reviewing procedure for this paper.

- **All: Identification of action points and follow up on exchange of information**

France (CG) can share historical data on rats. EFSA is interested to receive these.

For the mutual benefit of all involved, it would be beneficial if mandates, documents and information would be shared among all. To this end EFSA plans to create a shared access internet repository (Sciencenet: EFSA extranet)

ACTION 1.1. EFSA creates a space on the EFSA-extranet for exchange of information

ACTION 1.2. EFSA uploads mandate from DG SANCO on the shared space on EFSA-extranet

ACTION 1.3. France sends EFSA data on historical data of rats for uploading on the shared space on EFSA-extranet.

3. Acronyms, stakeholders, links to key files or URLs etc

1. Acronyms

Acronyms	Definitions
AF	Advisory Forum
AFSCO	Advisory Forum & Scientific Cooperation
AMT	Advice Management Team
ANSES	Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail
BfR	Federal Institute for risk assessment, Germany
BMELV	Federal Ministry of Food, Agriculture and Consumer Protection
EDIT	Editorial and Media Relations
EMRISK	Emerging Risks Unit
HCB	Haut Conseil des Biotechnologies
MS	Member States
MTG	Meeting
NVWA	Netherlands Food and Consumer Product Safety Authority
OECD	Organisation for Economic Co-operation and Development
RIKILT	Institute of Food Safety, Netherlands
RIVM	National Institute for Public Health and the Environment, Netherlands
RT	Response Team
SAS	Scientific Assessment Support Unit
TC	Teleconference

2. Links and URLs to Key files

A new science project was created to allow exchange of information between EFSA and MS. It is accessible here:

Collaboration Document Folder: **Seralini Study EFSA & MS**

https://sciencenet.efsa.europa.eu/portal/server.pt/gateway/PTARGS_32_0_229_0_-1_47/http:/bea-aps.efsa.eu.int:11930/collab/do/document/overview?projID=848332&folderID=848336

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4. Addedum

Full text of The Netherlands (NVWA) Preliminary Conclusions

Mandate

The Netherlands Food Safety Authority (NVWA) will next week deliver its scientific review of a new study which states concerns over the potential toxicity of a herbicide containing glyphosate and of glyphosate-tolerant GM (NK603) maize. Besides representatives of the Office for Risk Assessment and Research (BuRO) of NVWA, a multi-disciplinary task force was set up by the institutes RIVM and RIKILT and has analysed the paper by Séralini et al. This work will address a request for scientific advice from the House of Representatives as forwarded to the Secretary of the Ministry of Agriculture (EL&I) and Secretary of the Ministry Environment (I&M) following by a general consultation "Pesticides" before October the 8th. NVWA has been asked to consider the significance of the study's findings of Séralini et al. as part of its ongoing statutory remit to monitor scientific developments relating to its work which is the assessment of food/feed safety and its related risks.

Preliminary conclusions

In general, the reporting of the research performed by Séralini et al. is very imperfect. Many details that are essential for a proper assessment are missing, such as for example the compositional analysis of the GM maize and control maize, the rodent diets, feed and water consumption or growth rate, and information about whether or not the study was blinded. A statistical analysis of amongst others, consumption, growth rate, mortality, and cancer incidence and multiplicity has not been carried out or included in the paper, and essential data about the statistical analysis of biochemical data that was carried out are missing.

1) The aim of Séralini et al (2012), as far as can be judged from the publication, was to carry out a chronic/long-term feeding trial using the usual parameters for a 90-day study (supplemented by some additional parameters). The setup of the study is complicated and does not follow clearly one of the OECD guidelines for laboratory animal research. Generally speaking, to be able to assess the carcinogenicity of a substance or product, the setup as selected by Séralini et al. is not suitable. The rat species chosen for this study - Sprague Dawley - is one strain with a high background incidence for certain types of cancer such as mammary gland tumours and pituitary cancer upon ageing i.e. the end of the rat's lifetime. A higher number of rats per experimental group than 10/sex/dose should have been used. Because of the small group size in this feeding study, the differences between the control and treated groups can be explained as being coincidence. It is all the more a major information gap that the publication does not include a statistical analysis of the effects of the various treatments on mortality and tumour incidence and/or has not been carried out. The conclusions of Séralini et al. related to tumours and the other reported effects therefore have no scientific basis.

More specifically, the number of animals, 10/sex/dose, is too low to be able to conclude whether or not an agent is carcinogenic. For a chronic toxicity study this number (n=10) is sufficient (OECD guideline 453), provided this is supported with data from the n=50 animals/sex/dose in the combined carcinogenicity study. "For a thorough biological and statistical evaluation of the study each dose group should at least contain 50 animals of each sex. Each dose group and concurrent control group intended for the chronic phase of this study (OECD 453 and not TG 452, which requires a higher number) should contain at least 10 animals/sex."

Additionally, the materials and methods section describes that 33% control maize and 33% GM (NK603) maize was processed into the standard rodent diet of respectively the control animals and the test animals as the highest dose. The animals that received the lower doses of GM (NK603) maize had a standard rodent diet in which respectively 11 and 22% GM-maize was processed. However, results of the controls fed with 11 and 22% control maize in their diet are missing in the paper. The ratio maize/standard rodent diet was therefore not equal in all animal groups. It may mean that effects could also be caused by these differences in diets which have nothing to do with the nature of the GM-(NK603) maize.

2) The publication of Séralini et al (2012) reports that 'currently, no regulatory authority requests mandatory chronic animal feeding trials to be performed for edible GMOs and formulated pesticides'. Whereupon reference is made selectively to some publications in which, according to the authors, adverse effects are found of inclusion of GMOs in the diet of experimental animals or in that case of Roundup formulations on cell cultures. The authors are completely ignoring the many (estimated more than 100) publications in which diets containing different percentages of GMOs are fed during 90 days or longer to experimental animals and where no adverse effects on health were found (Snell et al. 2007). The remark by Seralini et al. that animal experiments so far are not required for substantially equivalent GMOs is correct, but there is no reason to change this. EFSA recommends conducting a 90-day feeding study with the whole GMO product only if appropriate. This is not the case when a GMO product is considered to be 'substantial equivalent' to a non-GM isogenic counterpart (EFSA, 2011).

On the other hand, in the same paragraph Séralini and co-authors suggested that, if studies were carried out according to the protocol as described in their new publication, chronic GMO feeding trials would demonstrate health effects. Although their study is not suitable to base conclusions on, this is also not a cogent argument to undermine the conclusions, for instance, from the study of Snell et al. (2007) .

3) According to the JMPR (2004) and the EC (2002), glyphosate and/or residues of glyphosate are not carcinogenic.

The herbicide with the commercial name Roundup contains as active substance glyphosate. Other constituents of the formulation are not listed. Based on the article of Séralini et al., it cannot be concluded that (residues of) glyphosate (in the form of Roundup*,) or the genetically modified NK603 maize alone, or the GM (NK603) maize in combination with Roundup or Roundup alone, are carcinogenic.

* It should be noted that the authors studied effects in rats resulting from exposure to a commercial product that contains glyphosate with the brand name GTplus, which is referred by the authors as being Roundup.

More specifically, the toxicity of the active substance glyphosate has been assessed in the EU in 2000 and in 2004 by JMPR. It was evaluated that glyphosate is not carcinogenic as could be concluded from well-conducted carcinogenicity studies in mice and rats. In addition, JMPR concludes that AMPA (the principal metabolite in plants) is less toxic than glyphosate. The ADI for glyphosate established by JMPR of 1 mg/kg bw/day is based on effects on the salivary glands as seen in chronic/carcinogenicity studies (NOAEL 100 mg/kg bw/day).

4) Séralini et al. have not demonstrated significant effects on mortality and tumour incidence, because a statistical analysis was not carried out. If a statistical analysis would have been carried out, than the usual analysis by pairwise testing of dose groups compared to control for these parameters would not have led to statistically significant differences. That is also not to be expected on the basis of a study design with such small numbers of animal per dose group. Because of the low number of animals per group, it is the very probable that the observed differences are based on coincidence.

More specifically, the number of animals with for example mammary gland and pituitary tumours did not increase clearly with increasing the dose of GM (NK603) maize or glyphosate in the drinking water. The authors argue that there is a 'threshold response ' triggered by an impact on the endocrine system. This is however not a scientifically sound conclusion, because thresholds with 10 animals per dose group will completely lie outside the statistically observable area.

With the small number of animals used, it is to be expected that the observed part of animals with an adverse response will be erratic, even if there is a normal dose-response relationship. Furthermore, it is considered highly unlikely that the GM (NK603) maize will have the same effect in rodents as the glyphosate formulation used.

As mentioned before the authors claim an effect on the endocrine system. From the description of the study (material and methods), it seems that the phase of the cycle of the female animals at the time of blood collection has not been taken into account. This, in combination with a too low number of animals per test group, can lead to differences in estradiol concentrations that may very well be based on coincidence.

To summarize, the study design, the way research was conducted and the reporting of the results are not suitable to be able to come to a science-based conclusion on the supposed non-dose related endocrine disruption. Such a conclusion would require many more animals and a proper statistical analysis.

Another concern relates to the biochemical changes observed between various test groups if compared to the controls. The author's conclusion is not verifiable: the underlying data are not included in the publication and the statistical method chosen for the data analysis, i.e. two class discriminant analysis, seemingly aims to find differences than rather testing whether there can be differences demonstrated in the measured biochemical parameters between treated animals and the control group.

Finally, the authors link treatment and effects that are not scientifically underpinned by their results observed in the long term feeding trial11.

HN (NVWA-BuRO)