Critical remarks on the long-term feeding study by Séralini et al. (2012)

Does the study provide proof of health threats posed by genetically modified foods?

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This article analyzes the long-term feeding study of Séralini et al.,[1] in which rats were fed for over two years with genetically modified maize NK 603 treated with and without Roundup Ready and drinking water containing Roundup. In the study the rats died earlier and developed more tumors than rats from a control group. However, these findings are questionable because there are serious methodical flaws and shortcomings in the representation of the data, and the published data do not allow an interpretation of increased cancer rates and premature death. Due to the low number of animals used in the test groups, it appears that these observed effects occurred by chance. The consequences of the study are discussed in respect of risk assessment of genetically modified organisms and the authorization process.

I. Introduction

Gene engineering, the implementation of scientific knowledge from genetic engineering and molecular biology into products, is now applied in many different areas of our economy and these products have become part of our life. However, under the term "gene engineering" most Europeans think almost exclusively of "green gene technology/plant biotechnology/agro gene technology", especially in Germany. Today, among all other applications green gene technology is regarded as dangerous and unnecessary. Profound scientific findings are hardly taken into account, but speculative assumptions and scientifically untenable assertions become the yardstick of action instead.

Liberation of genetically modified organisms (GMO) and/or placing on the market are strongly regulated by the Novel Food Regulation[2] (1997–2003) and also by the Regulations (EC) No 1829/2003,[3] (EC) No 1830/2003,[4] and Directive 2001/18/EC.[5] The organisms and products derived from GMOs must be safe. In order to prove the safety toxicological tests must be carried out by the applicants, and a risk assessment must be performed by the European Food Safety Authority (EFSA). Only organisms and/or products that are evaluated as safe by EFSA can be authorized by the EU Commission. Of course, the toxicological tests, including animal feed trails, are not specified in detail in the above mentioned regulations; however, these are described comprehensively in corresponding guidance documents prepared by EFSA.[6] However, the guidance document only requires "animal feeding studies

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1 G.-E. Séralini, E. Clair, R. Mesnage, S. Gress, N. Defrage, M. Malatesta, D. Hennequin, J. Spiroux de Vendomois, "Long term toxicity of a roundup herbicide and roundup-tolerant genetically modified maize." 50 *Food Chem. Tox.*" (2012), pp. 4211.

2 Commission Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ 1997 L 43/1.

3 Commission Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ 2003 L268/1.

4 Commission Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. OJ 2003 L268/24.

5 Council Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the liberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ 2001 L106/.

6 EFSA Panel on Genetically Modified Organisms (GMO), "Guidance for risk assessment of food and feed from genetically modified plants." 9(5) EFSA Journal (2011), p. 2150.

with whole food and/or feed from GM plants when considered necessary." When regarded as necessary, feeding trails should be performed according the OECD guidelines[7] for testing chemicals. It is not specified whether a 28-day or a 90-day oral toxicity feeding trial in rodents should be performed. It depends on the toxicological endpoints (sub-chronic, chronic toxicity, cancerogenicity).

During the last decade strong public and scientific discussion has influenced the concept of substantial equivalence and the duration of the feeding trials. In particular, there was debate about whether 28-day and 90-day feeding trials are sufficient for assessing the safety of GMOs and products derived from GMOs[8,9] or if long-term feeding studies or even multi-generation tests would be more adequate[10] and should therefore be mandatory.

Séralini et al. published in September 2012 a long-term feeding study in rats with genetically modified (GM) maize NK 603 and Roundup formulations in the journal *Food and Chemical Toxicology*. Their findings are cause for concern: rats die earlier and at higher rates and develop earlier and more tumors due to the inclusion of GM maize NK 603 and/or Roundup formulations in their diet. These findings are observed after feeding the animals for more than 90 days. However, just 90-day feeding studies are often used to assess the safety of GM products and GM plants. The GM maize NK 603 was evaluated by EFSA[11] (2) as just as safe as conventional maize and approved by the European Commission in 2004 as feed[12] and in 2005 as food[13] to market. Here arises the question whether the approvals were illegal due to an inadequate safety assessment by EFSA.

The results from the long-term feeding study attracted large media attention as well as the interest of NGOs and the scientific community. Gene technology critics and opponents[14,15] (5, 6) saw here the long-awaited scientific proof of the danger of foods from GMOs as solely result driven. Since commercial actors may not place unsafe foods on the market, they must be banned.

In this contribution methodological flaws and shortcomings of the feeding study are shown, and its implications with regard to the risk assessment of GM maize NK 603 maize and Roundup formulations are discussed.

II. Toxicological studies of food products in animal feeding trials

Foods as a whole were evaluated toxicologically only rarely in the past due to their complexity (large number of known and unknown ingredients) with regard to their safety. This changed rapidly with the introduction of food irradiation. To identify possible radiation-induced toxic effects, appropriate animal feeding trials were conducted.[16] Such investigations have led to the risk assessments of "novel foods" (e.g. new technologies in food processing)[17] and transgenic plants and foods produced from them (transfer of genes, unexpected effects). The

7 OECD (1998) "OECD guidelines for testing of chemicals – repeated 90day dose oral toxicity study in rodents, 408" http://browse.oecdbookshop.org/OECD/PDFs/free/9740801e.PDF.

8 EFSA, "Report of the EFSA GMO Panel Working Group on animal feeding trials: safety and nutritional assessment of GM plants and derived food and feed: the role of animal feeding trials". 46 *Food Chem.Tox*. (2008), p. S2.

9 EFSA Scientific Committee, "Guidance on conduction repeated-dose 90-day oral toxicity study in rodents on whole food/feed" 9(12) *EFSA-Journal* (2011) p. 2438.

10 G.-E. Séralini, R. Mesnage, E. Clair, S. Gress, J.Sprioux de Vendomois, D. Cellier, "Genetically modified crops safety assessments: present limits and possible improvements" 23 *Environmental Sciences Europe* (2011), p.10.

11 EFSA "Opinion of the Scientific Panel on genetically modified organisms on a request from the Commission related to the safety of foods and food ingredients derived from herbicide-tolerant genetically modified maize NK603 maize, for which a request for placing on the market was submitted under article 4 of the novel food regulation (EC) No. 258/97 by Monsanto." 9 *The EFSA Journal* (2003), p. 1.

12 Commission decision of 19 July 2004 concerning the placing on the market of a genetically modified maize product glyphosate (Zea mays L. line NK603) pursuant to Directive 2001/18/EC of the European Parliament and of the Council. 295 OJ (2004), p. 35.

13 Commission decision of 3 March 2005 on the authorization of the placing on the market of the genetically modified maize line NK 603-derived foods and food ingredients as novel foods or novel food ingredients under Regulation (EC) No 258/97 of the European Parliament and of the Council. 2005 OJ 158, p. 20.

14 ENSSER (European Network of Scientists for Social and Environmental Responsibility), "Questionable biosafety, double standards and, once again, a 'Shoot-the-Messenger' style debate", Statement on Séralini et al. (2012) publication and reactions evoked in Hyderabad, India at COP-6 meeting (report): http://XA.yimg.com/KQ/groups/18208928/312025102/name/ENSSER_Seralini.pdf.

15 Greenpeace, Corinne Lepage et Gérard Bapt, (2012): "OGM et agriculture – corn OGM: reactions", http://www.corinnelepage.EU/mais-OGM-reactions-de-Greenpeace-Corinne-Lepage-et-Gerard-BAPT .

16 P. S. Elias, (1980) "The wholesomeness of irradiated food." 4 Ecotoxicol. Environ. Saf. (1980), p. 172.

17 See supra, note 2.

problems that already existed in the feeding experiments with 'irradiated' food still exist today; they are due to the physiology of the animals and the complexity of the composition of the substance 'food'. Great experiences and knowledge exist for the toxicological testing of individual substances (e.g. flavorings, colorings and additives). These mostly chemically-defined single substances can be easily added in sufficiently high amounts to the diet without changing the nutritional value of the diet which may lead to nutritional imbalances. Diet-related effects can be largely excluded. Observed/measured changes/effects can be mostly attributed to the test substance, and often involved metabolic pathways can be traced. Due to the usable, distinct doses, dose-effect-relationships can be observed, and important parameters (e.g. NOEL) can be determined. The case is quite different with complex composite foods, which contain many different macro and micro substances. Therefore, accurate and comprehensive chemical analysis is essential for the test and reference food. The extensive knowledge of the chemical composition is a prerequisite for such toxicological studies. In general, the test food cannot be added in sufficiently high quantitis to the standard diet. Nutritional imbalances can occur easily and observed effects are not attributable to the test food, but are rather due to the diet. Acute effects can be hardly caused. Many metabolic pathways are affected/involved due to the large number of food components, and the observed effects, if any, cannot be attributed to the test food.

III. Séralini long-term feeding study: Experimental design

In this long-term feeding study the toxicity of products derived from GMOs (here gm maize NK 603) and of two formulations of Roundup (Weather-Max and Roundup GT plus) should be investigated. The research group of Séralini fed rats (Harlan Sprague-Dawely) with GM maize NK 603 and added Roundup GT to their drinking water for over two years. In total 200 animals were fed and typically *ad libitum*. Nine test groups were compared with a control group. The control group, which included 10 females and 10 males, was fed with standard diet A04 containing 33 % conventional maize and "normal" drinking water. Six additional groups consisting of 10 females and 10 males each were fed with GM maize NK603 (11, 22, 33 % in the standard diet) and GM maize NK 603 (11, 22, 33 %) treated with Roundup Weather-Max during cultivation. Three additional groups received 33 % of conventional maize, but this time the drinking water contained 1, 1 % x 10^{-8}, 0.09 %, and 0.5 % Roundup GT plus. According to data by Séralini et al.[18] these percentages correspond to levels of 0.00005 mg/L, 400 mg/L and 2250 mg/L glyphosate. The dosage was chosen deliberately; they should demonstrate effects that might occur by typical applications/residues of Roundup in agriculture and house gardens. Over the entire study period, the survival time, death and the occurrence of tumors or pathological diseases were observed and registered, respectively. After the end of the trial animals were dissected, and the tumors were analyzed histo-pathologically/morphologically. Food and water intake and body weight of the individual animals were measured. 47 parameters were determined in the blood and urine samples 11 times; more than 500 datasets have been obtained.

VI. Critical comments

1. Methodological shortcomings

Sprague-Dawely rat strains (Harlan, Charles River) are often used in toxicological studies as these strains are well characterized and genetically uniform, so that effects can be better analyzed and compared. However, both strains tend to develop spontaneous tumors[19,20,21], and this increases if there is no food restriction[22] (*ad libitum* feeding, as performed in the Séralini study). However, there are differences between the two strains in respect to the morality and tumour incidence. Such differences

18 See supra, note 1.

19 G. Bode, F. Hartig, G. Halliday, H. Czerwek, (1985): "Incidence of spontaneous tumors in laboratory rats." 28 Experimental Pathology, (1985), p. 235.

20 M. Nakazawa, T. Tawaratani, H. Uchimoto, A. Nanda, M. Ueda, A. Ueda, Y. Shinoda, K. Iwakura, K. Kura, N. SUMI, "Spontaneous neoplastic lesions in aged Sprague-Dawley rats." 50 *Experimental animals* (2001), p. 99.

21 H. Suzuki, U. Mohr, G. Kimm, "Spontaneous endocrine tumors in Sprague-Dawely rats." 95 *J. Cancer Res. Clin. Oncology* (1979), p. 187.

22 M.N. Ross, G. Brass, "Cancer incidence patterns and nutrition in rats". 87 J. Nutrition (1965), p. 245.

have to be taken into account for long-term feeding studies. In addition, the OECD guidelines, 451[23] or 453,[24] which recommend 50 animals/sex and groups, should be applied for studies on the chronic toxicity and cancerogenicity. Instead, the Séralini study followed the OECD guideline 408[25] for 90-day studies with 10 animals each. Regardless of all the OECD recommendations[26] in the Séralini study, only 20 animals were used as a control group compared to 180 animals in the test groups. This number is far too low for such a long-term feeding study. This must be seen against the background that the rat strain "Harlan" increasingly leads to the formation of spontaneous tumors during lifetime. Even the breeder from whom the rats were obtained describes in his information about their life expectancy and spontaneous disease:[27] "... pituitary gland tumor were found in 20 % of the males and 39 % of the females. This relatively low incidence had little effect on the survival of the females (50 %) due to the high incidence (76 %) of mammary gland tumor (predominantly fibroadenomas)". Already against this known background the findings in the test groups are doubtful and questionable in respect to the low number of animals in the control group. Séralini[1] claimed repeatedly that he would not have expected either tumors or cancerogenicity, and therefore he started the study with only 10 males and 10 females per group. This is surprising as in other studies[28,29] on glyphosate and Roundup formulations, he described the cancerogenic effects of the compounds, and he also suspected such effects from products derived from GMOs.[30] It was pointed out[1] that the feeding study was originally scheduled as a 90-day study, but as increased toxic effects were encountered, the feeding was extended to over two years. From the publication, however, such effects cannot be recognized. Only in the male test group that was fed with 11 % GM maize are a spontaneous death and tumor visible after 105 days of feeding. It was not justified why just this single individual case gave rise to the expansion of the feeding time. According to the OECD guidelines, for a meaningful toxicological study on cancerogenicity a feeding experiment with a new design should have been carried out.

Overall the experimental design chosen by Séralini must be regarded from the known facts[31,32] i.e. the strain and limited number of animals per sex and group was inappropriate to ensure statements about the toxicity/cancerogenicity of the test substances GM maize NK 603 and Roundup formulations.

Detailed knowledge of the chemical composition of the feed or food is one of the basic prerequisites for toxicological studies in feeding experiments. The need for such investigations has been intensively discussed and evaluated.[33,34,35] Séralini et al. gave no information about macro- and micro-nutrients or other components of the feedstuff. Also, comparative chemical analysis of the conventional reference maize to the GM maize NK603 is missing.

23 OECD guideline (2009): carcinogenicity studies, OECD guidelines for the testing of chemicals, 451; http://www.oecdbookshop.org/OECD/display.asp?lang=en&sf1=identifiers&st1= 5lmqcr2k7mq4.

24 OECD (2009): OECD guideline for the testing of chemicals, COMBINED chronic toxicity/carcinogenicity studies, 453. http://www.oecd-ilibrary.org/docserver/download/fulltext/9745301e.pdf? 1348710784 = expires & id = id & accname=freeContent&checksum=B952BFD6EE1A7D91C0FA5FC33797F237.

25 See supra, note 7.

26 OECD (2009) OECD guidelines: http://www.OECD-ilibarary.org/environment/OECD-guidelines-for-testing-of-chemicals-section-4-heatth-effects_2074588.

27 Harlan Sprague-Dawely: http:// www.harlan.com/download.axd/117b20f991764a5e98e32d36 6d83e876.PDF? d=spraguedawley%20.

28 N. Benachour and G. E. Séralini, (2009) "Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells." 22 *Chem. Res. Toxicol.* (2009), p. 97.

29 C. Gasnier, C. Dumont, N. Benachour, E. Clair, M. C. Chagnon, G. E. Séralini, "Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines". 262 *Toxicology* (2009), p. 184.

30 J. Spiroux de Vendomois, F. Roullier, D. Cellier, G. E. Séralini, (2009) "A comparison of the effects of three GM corn varieties on mammalian health." 5 Int. J. Biol. Sci. (2009), p. 706.

31 B. S.Wahle, G.K. Sangha, S.G. Lake, L.P. Sheets, C. Croutch, W.R. Christenson, "Chronic toxicity and cancerogenicity testing in the Spraguae-Dawely rat of a prospective insect repellent (KBR) using the dermal route of exposure." 142 *Toxicology* (1999), p. 41.

32 J.C. Petersen, R.L. Morrissey, D.R. Saunders, K.L. Pavkov, I. Luempert, J.V. Turnier, D.W. Matheson, D.R. Schwartz, "A 2-year comparison study of CRL: CD BR and HSD: Sprague-Dawley SD rats." 33 Fund. Appl. Toxicol. (1996), p. 196.

33 See supra, note 6.

34 G. Flachowsky, H. Creates, U. Meyer, "Animal feeding studies for nutritional studies and safety assessment of feeds from genetically modified plants: A review." 7 *J. Verbr. Lebensm.* (2012), p. 179.

35 R.A. Herman, "Unintended compositional changes in genetically modified (gm) crops: 20 years of research." *J. Agri. Food Chem.* (2013) DX.DOI.org/10.1021/jf400135r.

Thus, it is not evident to what extent both feedstuffs are actually nutritionally and chemically equivalent. The publication does not clearly indicate what the addition of GM maize NK 603 (11 %, 22 % and 33 %) to the standard diet N04 really means. It does not show that these mixtures of diets are equivalent in their nutritional value. Since this information is completely missing, it must be assumed that three test diets with different nutritional values and chemical compositions were used. From a scientific view, only the results of 33 % maize groups should be compared. The lack of information about equivalence of the three diets makes it almost impossible to interpret the results derived from the different mixtures of diet.

Although the toxicity of foods containing Roundup formulations or glyphosate should be evaluated, the residual amount of glyphosate is not indicated in GM maize NK603 treated with Weather-Max. Also, information is missing about the application or the treatment times of GM maize NK603 with Roundup or other plant protection agents during cultivation. It is well-known that the toxicological responses of glyphosate and Roundup formulations differ widely, but throughout the publication glyphosate is often equated with Roundup.

The study compares two completely different Roundup formulations. In the drinking water test Roundup GT-plus was fed (450 g/L glyphosate is (51 % glyphosate, 7.5 % of a single substance, residual water), while the herbicide Roundup Weather Max 540 g/L glyphosate (48.8 % glyphosate and 51.2 % other substances) was used in GM maize NK603 test groups. A comparison of the results from drinking water test groups with those of GM maize NK603 with unknown glyphosate content is therefore limited. The results of test groups must be considered separately.

The selection of doses hardly corresponds to the reality for the lifetime oral ingestion of Roundup in drinking water.

Many biochemical parameters per test group were analyzed and approximately 500 datasets were created. A proper statistical analysis could have been possible for the individual animals. Instead, the Orthogonal Partial Least Squares Discriminant Analysis (OPLA) was applied, although it is not suitable in this particular case. Furthermore, the evaluation was carried out for only one test group (33 % GM maize NK 603, females) at the fifteenth month.

2. Results

The main objective of this study was to assess the potential toxicity of GM food (here maize NK 603) and formulations of the herbicide Roundup. However, nearly zero or only few evaluable data are presented. Raw data can only be taken from Figures 1 and 2. Due to the presentation style, the lifetimes of the animals and the occurrence of death or tumors can only be estimated ± 10 days. Diagrams showing the growth (weight increase) or feed and water intake are not given. However, just these data would provide supporting information on the health status of the animals. Information about increased tumors is incomplete, and the selection of the anatomical pathologies (Table 3) is rather subjective. The biochemical data used in the OPLA-DA statistic (Fig 5A) are not shown. Therefore, neither an evaluation of presented data (Fig 5A) nor the applications of other statistic methods is possible.

Although the biochemical data are used to underpin the claims about the diseases and tumors, no further information about all the other test groups is presented. Instead, to publish complete and robust data, almost half a page of color photographs of animals with very large tumors is shown. The pictures have nearly zero scientific "value" since the study does not provide photographs of the control groups, which might demonstrate whether it found similar tumors therin. Unsurprisingly, in the Séralini study exactly the same tumors are found with nearly the same frequency as indicated by the breeder from his long-term experience with the "Harlan" strain.

3. Lifetime/mortality

The authors describe quite succinctly that approximately 50 % of the male and 70 % of the females in the test groups died prematurely before reaching the normal life expectancy (about 2 years) compared to the animals in the control groups. The authors regarded this as clear proof of the toxicity of GM maize NK 603 and Roundup or glyphosate, respectively. This consideration is neither appropriate nor valid. It does not account for either possible dose effects or differences from the drinking water in the GM maize NK603 test groups. Also, the reference point is questionable in respect to the lifespan of females in the control group. In the Séralini

study, only two deaths occured in the female control group, and thus the life expectancy of the females was exceptionally high – 80 %. The historically found values are, however, at an average of 42 %[29]. Probably this high lifespan observed in the Séralini study is purely by chance and arises merely from the insufficient number of animals. The lifespans of the male control animals are within the range of historically known values. A separate analysis of mortality and survival times in the test groups would be appropriate. The lifespan of the animals in the drinking water, the 33 % GM maize NK603 with and without Roundup test groups is represented in a slightly different form in Figures IA and IB. To avoid confusion, figures and tables are listed with Roman numbers in this report.

4. Roundup in drinking water

Doses of 50 ng/L, 400 mg/L and 2250 mg/L of glyphosate were applied in the drinking water test. A dose effect should be expected due to the wide range of concentrations of the active substance. However, for a reliable measurement of *low dose effects* or inverse effects it is insufficient to use only one low dose.

A negative influence of Roundup GT Plus on the lifespan is hardly ascertainable in males. At lower concentrations of glyphosate mortality, the lifespans are not significantly different from those of the control group. In contrast, the highest concentration of glyphosate (2.25 g/L) has even a positive effect; fewer animals die and a prolongation of the life time can be observed of almost 100 days (Fig. IA). This high concentration of glyphosate seems also to counteract the development of tumors. At the lower levels no significant influences are visible either on life or death (Fig. II). If this observation is accepted as a true fact, than this could be interpreted that Roundup GT Plus or glyphosate has protective effects on the lifespan of male rats. A dose-response relationship is not detectable, but also no indication of *low dose effects* can be identified.

Females die earlier in all three test groups (Fig. 1A) compared to the control group. However, the reason for this finding is only due to the high survival rate of the rats observed in the Séralini study. Surprisingly, the life times of the test groups correspond almost to the historical data for "untreated" rats. Again, dose effects are also not observable. Apparently, the herbicide has no biological relevance on the survival rate. The small differences between the three test groups are not significant.

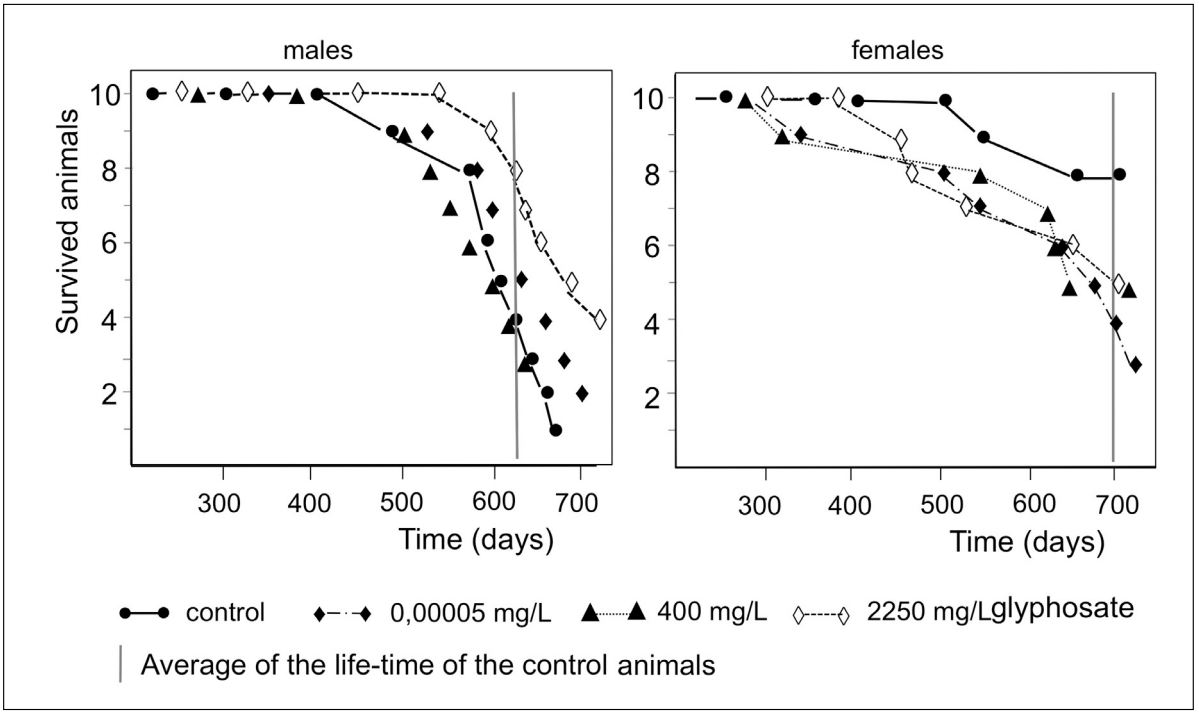

Fig.: IA Lifetime of the rats in the drinking water test group

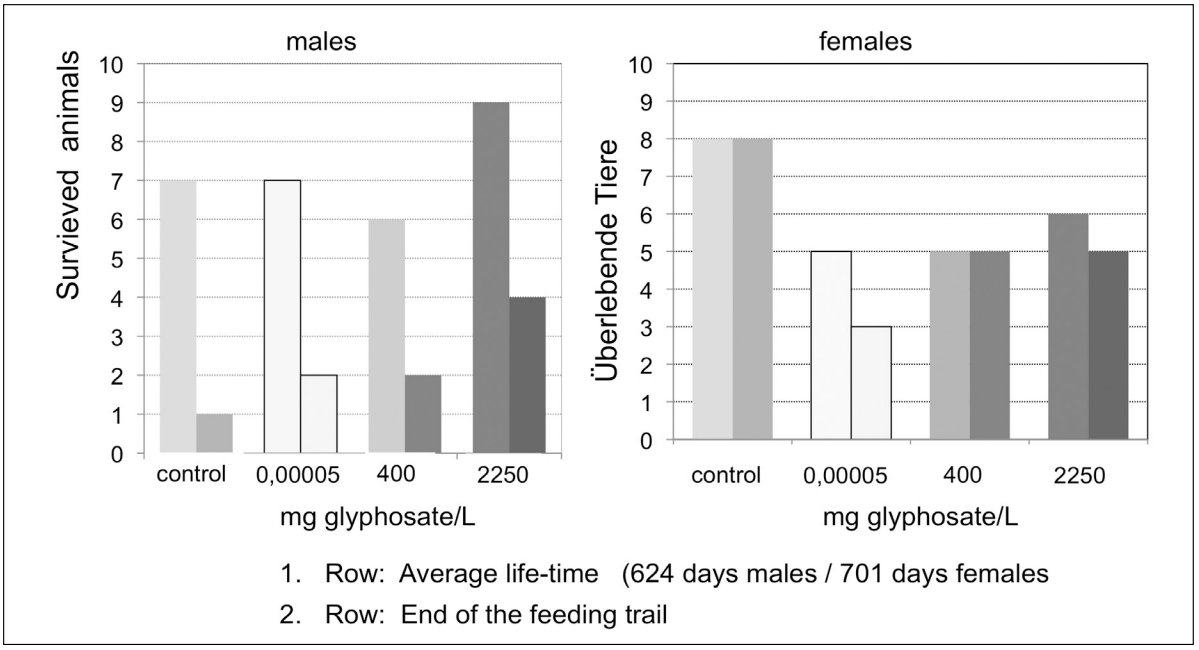

Fig.: II Surviving animals in the drinking water test group

Viewed across the entire drinking water groups (males, females), five animals survives in the control group as well as in the test groups with the highest glyphosate concentration. Almost all the males die independent of the amount of glyphosate at an old age almost without striking tumors, whereas females die from around the 300th day and had to be killed due to ethical reasons.

Seven out of ten male animals survived in the control group. However, 9 out of 10 animals survived in the test group with 2.25 g/L glyphosate. At the lowest concentration (50 ng glyphosate/L), 7 animals (2 spontaneous deaths; 1 killing) died as well as in the control group but with prolonged life, at least about 50 days (Fig. IA and II).

No female animals died spontaneously, and all animals had to be killed as a result of heavy tumors. This also applied to the control group. Considering the deaths as a whole, no significance or biological relevance of Roundup RT Plus is really measureable.

5. Maize NK 603 with and without Roundup treatment

Due to the uncertainty of the comparability of the different diets, the findings resulting from the test groups fed with 33 % GM maize with and without Roundup treatment are considered at first. The differences in survival rates are not so pronounced as in the drinking water test groups (Fig. IB). But also in the male test groups, the survival rate is higher (approximately 60 days) independent of whether the maize was treated or not with Roundup as com-

NK 603 %	Male		Female		Round-up NK 603	Male		Female	
	spontaneous	killed*	spontaneous	killed		spontaneous	killed	spontaneous	killed
0	3	0	0	2	0	3	0	0	2
11	4	1	1	2	11	3	1	1	3
22	1	0	1	6	22	2	3	1	6
33	0	1	0	4	33	3	0	1	3
Data were taken from Figure 1 of the Séralini study. Animals were killed prematurely due to the size of the tumors.									

Table: I Deaths in the test group GM maize NK 603 with and without Roundup treatment

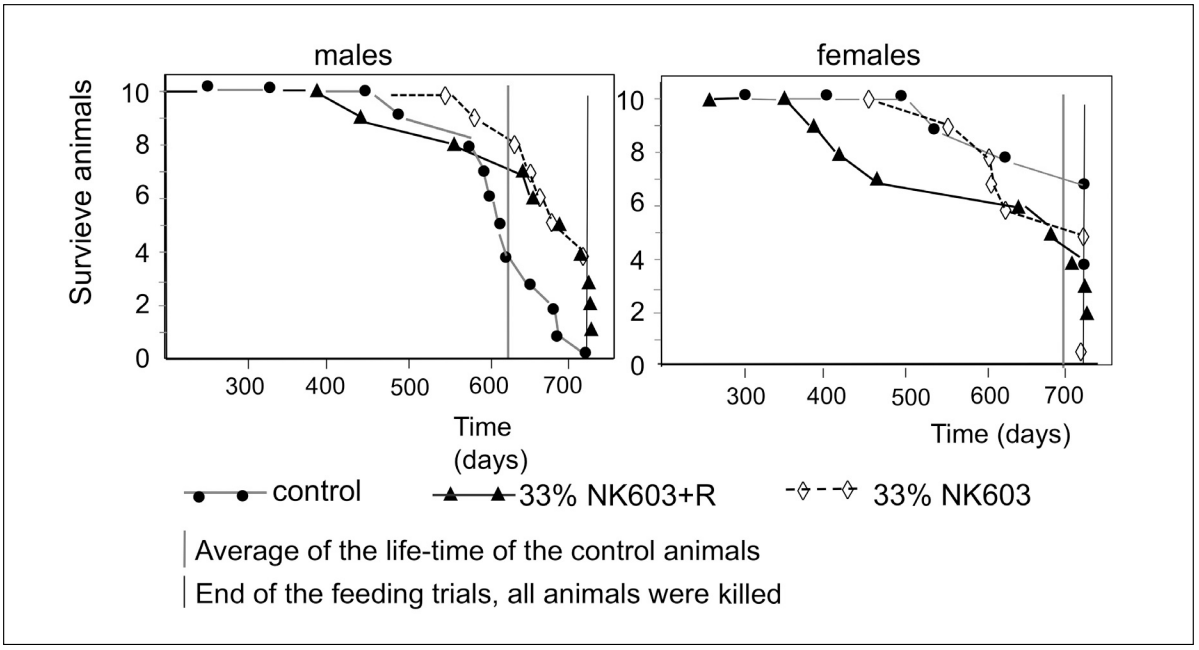

Fig.: IB Lifetime of the rats GM maize NK 603 test group

pared to the control group. There are no statistically significant differences in the deaths. Three animals died in the control group as well as in the test group for GM maize NK 603 with Roundup treatment, whereas only one animal died in the test group GM maize NK 603 without Roundup treatment (Tab. 1). The female animals revealed no significant differences in survival rates, however, in the test group GM maize NK603 with Roundup treatment the animals died earlier than in the control group. In the control group two deaths occured while four deaths happened in both test groups GM maize NK603 with and without Roundup treatment.

In feeding test groups with 11 % and 22 % GM maize dose-dependent effects might be surmised. Feeding of 11 % GM maize with and without Roundup treatment resulted in the highest negative impact on survival time and mortality rates for males. These effects are not identifiable for females. The intake of 11 % and 22 % GM maize NK603 has a lower impact than the 33 % GM maize NK 603. A negative inverse effect might be assumed for males; the lower the GM maize content (with or without Roundup) the higher the negative influence on life and mortality.

Statistical analyses[36,37] of the published data according to Kaplan-Meier reveals that the observed differences are not significant between the test and control groups. The raw data do not support the statements by Séralini about higher mortality rates and shorter lifespans of rats due to GM maize NK 603 or of Roundup. The observed findings are randomly occurring events, which are typical for the rat strain used.

6. Tumors and other pathological diseases

Raw data for the temporal occurrence and the numbers of tumors can be withdrawn from Fig. 2. The most frequently observed anatomical pathologies are presented in Table 3. As shown in Figure 2 in the control groups, no tumors occured within the first 420 days, while at the same time in the test groups already 10–30 % of the females had developed tumors. On the other hand, for example, in

36 ESFA final review of the Séralini et al. (2012a) 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. 10(1) *EFSA Journal* (2012), p. 2985; http://www.efsa.europa.eu/en/efsajournal/doc/2986.pdf, and Annex 1, http://www.efsa.europa.eu/en/efsajournal/doc/2986ax1.pdf.

37 Opinion of the French Agency for Food, Environmental and Occupational Health & Safety concerning an analysis of the study by Séralini et al. (2012) "long term toxicity of a ROUNDUP herbicide and a ROUNDUP-tolerant genetically modified maize" ANSES (2012), http://www.anses.fr/documents/BIOT2012sa0227EN.PDF.

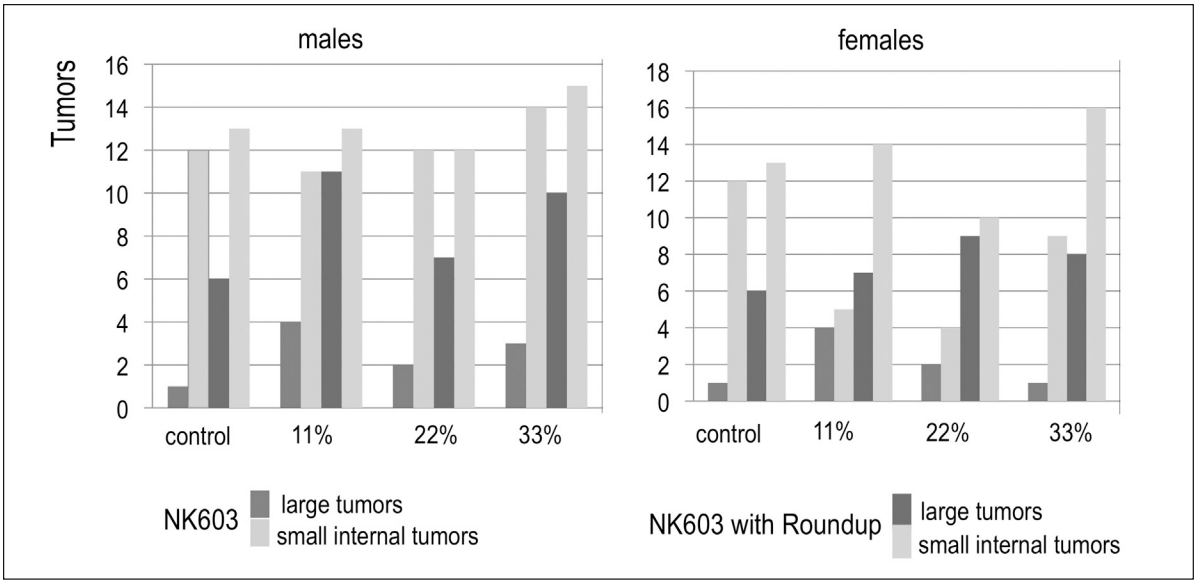

Fig.: III Number of tumors in rats developed in the test groups after feeding GM maize NK 603 with and without Roundup treatment.

males tumors first occured at the 520th day in the drinking water test group with 2.25 g glyphosate/L. However, this generalizing approach is not applicable. Again, the data must be analyzed separately and sex-specifically within the test groups. It is apparent that no dose-response relationship exists. Almost entirely, tumors occur earlier at lower concentrations of glyphosate in drinking water or at lower quantities of GM maize NK 603 than at higher doses. As for mortality, females are apparently more sensitive to GM maize NK 603 and to Roundup due to their generally longer lifespan. Again, no significance can be proven for the observed tumors within the test groups. The total number of tumors varied only slightly compared to the control animals. In the male control group 13 tumors developed, while 7, 9, and 10 tumors, respectively, occured in the test group 11 %, 22 % and 33 % GM maize NK 603 with Roundup treatment. The number of large tumors, as also shown in Figure 1, varied between one and three. Not surprisingly, more tumors occured in females. 19 tumors were found in the control group, while 21, 19, and 24 tumors were reported after feeding diets of 11 %, 22 % and 33 % GM maize NK 603. The number of heavy tumors varied between 6 and 9 over all groups (control and test) (Fig. III). These minor differences cannot be regarded as significant due to the low number of animals used in the groups. An influence of the diet on the formation and increasing number of tumors cannot be deduced from the published data.

The most frequently observed anatomical pathologies on organs are listed in Table 3. The authors neither differentiate in the severity of the disease nor in the organ specificity. A dose dependency is not recognizable; they might be by chance spontaneously occurring diseases. A compilation of breast tumors and disorders of the pituitary gland and kidney across all groups is shown in Figure III. The fluctuation margin of the diseases is minimal; it varies between one and three diseases.

Maybe certain dose dependence could be derived in some cases, even if it is contradictory (Fig. IV). A positive dose-relationship might be assumed for breast tumors when testing Roundup treated GM maize NK 603, but such a dependency is not detectable for all other diseases.

GM maize NK603 probably has an inverse dose dependence for pathologies of pituitary glands, and an inverse effect can be detected in the kidney as a result of Roundup GT Plus in the drinking water. Here, maybe a threshold triggers the emergence of the diseases. Overall, such an interpretation is certainly not scientifically justified due to the low numbers of animals and doses used. The listed diseases are just the same ones as also found in the same frequency after long-term feedings of this strain with the normal standard diet. Again, the

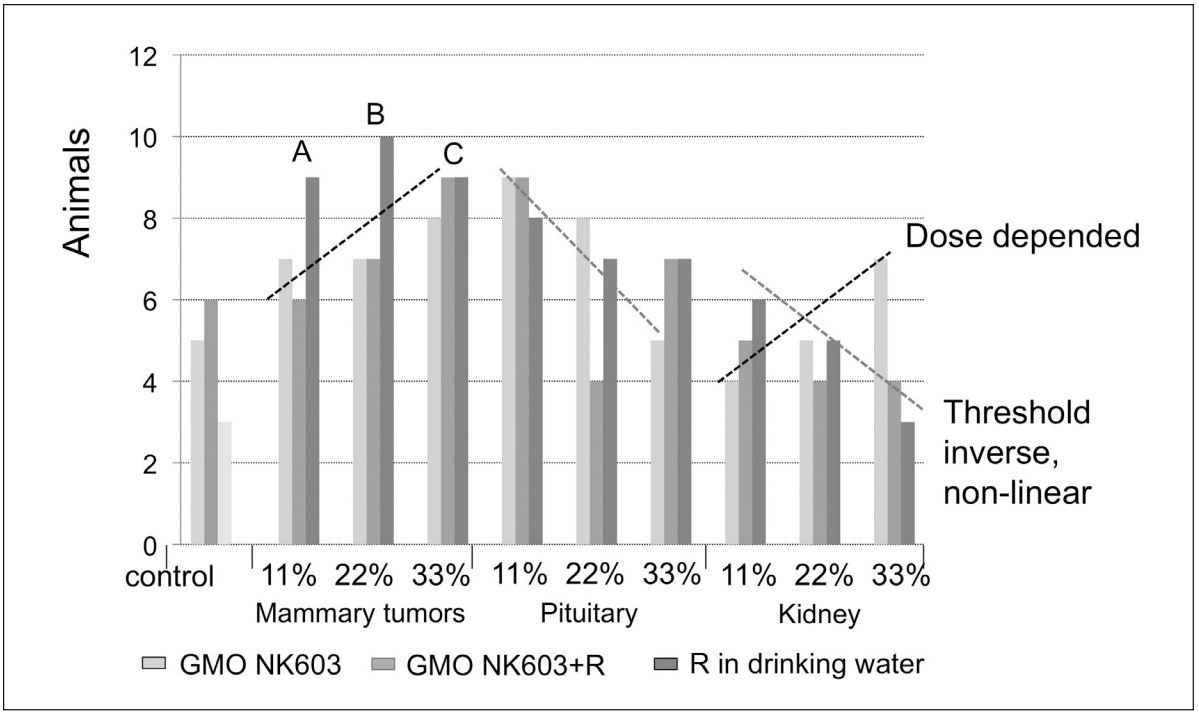

Fig.: IV Interpretable dose dependence effects on diseases A 50 ng/L, B 400 mg/L, C 2250 mg/L glyphosate in drinking water

influence of the test diets, GM maize NK 603 or Roundup, on increased tumor formation is not sufficiently covered in the published data.

7. Biochemical parameters

In the study, 47 blood and urine parameters were analyzed per animal of all groups at 10/11 different times. However, only the data are published in a recalculated manner from the females of the test group GM maize NK 603 at the time of the fifteenth month. Although the authors just use the changes in the biochemical parameters to support their statements about mortality and tumor occurrence, it is not comprehensible why only the dataset obtained from the fifteenth month was subjected to the OPLA statistical method, which is here unsuitable for this small number of data. The real experimental data have not been published. Thus, an evaluation is not possible. No statisticaly significant differences can be deduced between the control and special test groups (5A). As well, the statement that the diseases are due to changes in the testosterone-estradiol serum levels is not covered by the shown data (Fig. 5B). The remark that an unknown substance in GM maize NK 603 or Roundup acts on the hormone regulation similar to endocrine disruptors is not covered by the published data. Furthermore, the study design and the doses are not suitable to detect hormonal effects as derived from endocrine disruptors.

V. Conflicts of interest?

The authors[38] declared that they have no conflicts of interest. However, they acknowlege the support of the Association of CERES, the Foundation "Charles Léopold Mayer pour le progress de l´homme" (FPH), the French Ministry of Research and the CRIIGEN. It remains open what can be understood by "support" of a 3.2 million Euros expensive feeding study. CRIIGEN is a gene engineering-critical organization. Séralini was a co-founder of this organization, and today holds the position of Chairman of the Scientific Advisory Board.[39] In the period between 2000 and 2010 Carrefour belonged to the Board of Directors of

38 See supra, note 1.

39 CRIIGEN: http://www.criigen.org/SiteEn/index.php?option=com_content &task=blogcategory&id=52&103 =Itemid.

CRIIGEN and CRIIGEN was supported financially for research work, including on GMOs[40]. According to current knowledge [41] the feeding study was funded with 1.5 million Euros from CERES and 0.9 million Euros from the Foundation "Charles Léopold Mayer pour le progress de l´homme". The origin of the missing 0.8 million Euros has not been documented. CERES and the Foundation are known by anti-gene engineering campaigns. It can be assumed from contributions of two newspapers[42, 43] that the funds are not directly given to Séralini but were available via CRIIGEN. At least for Séralini the declaration of no conflicts of interest is more than questionable.[44]

VI. Concluding remarks

Only a few raw data are published in the feeding study by Séralini et al.[45] The statements of authors on mortality rates as well as the induction of tumors and kidney and hepatic diseases are due to the consumption of GM maize NK 603 and/or Roundup formulations are not covered by the limited data. The presented data allow no statements about negative health effects of GM maize NK603 with or without Roundup treatment. This scientifically insufficient research cannot be taken as an indication for a renewed assessment of GM maize NK 603. From a scientific view, also the publication does not justify a redesign of feeding studies (extension to two years) for safety assessment of transgenic plants or foods derived from GMOs.

More than 37 governmental authorities and scientific bodies [46,47,48] have analyzed the few available data. Their evaluations resulted in the statement that this study does not correspond to scientific criteria and, in its present form, is not suitable for a new assessment either of GM maize NK 603 or glyphosate or Roundup, respectively.

The regulation for the authorization process will be changed, and the EFSA guidance document for the risk assessment of GMOs and products derived from GMOs will be included in a regulation. The most important change is that 90-day feeding trials on rodents will be obligatory.[49] This decision may also have been driven by the long-term feeding study of Séralini et al.;[50] however, the requirement for a 90-day feeding trial is not scientifically justified, especially for GMOs of the first generation. Furthermore, obligatory 90-day feeding trials contradict efforts to reduce, refine and replace animal studies.

40 Carrefour: http://www.ISA-Conso.fr/Auchan-a-participe-au-financement-de-l-Etude-du-crriigen-sur-les-OGM.13294 and http://www.lexpress.fr.Actualite/Sciences/Sante/Auchan-et-Carrefour-Ont-aide-a-financer-l-Etude-sur-ogm_1164587.html.

41 Funding: http://PRG-Poitou-Charentes.org.

42 Le Nouvel Observateur 2012 "Oui, les OGM sont of the poisons!" http://tempsreel.nouvelobs.com/OGM-Le-scandale/20120918.OBS2686/EXCLUSIF-Oui-Les-OGM-sont-des-poisons.html.

43 C. LePage (2012), "Corinne Lepage (MEP) on why the Séralini of study is a 'bomb'. A review and historical approach." http://www.huffingtonpost.fr/Corinne-Lepage/OGM-une-Etude-et-une-demarche-historiques_b_1907658.html?utm_hp_ref = france.

44 "Etude Anti-Ogm de Séralini: les petits soldats de la Fondation pour le progress de l homme". http://alerte-Environnement.fr/2012/11/12/Etude-anti-OGM-de-saralini-Les-Petits-soldats-de-la-Fondation-pour-Le-Progres-de-Lhomme/.

45 See supra, note 1.

46 See supra, notes 32 and 34.

47 Academies Françaises "Avis des Académies national d'agriculture, de médecine, de pharmacie, of the sciences, of the technologies, et Vétérinaire récente sur la publication de G.E. Séralini et al. sur la toxicité d'un OGM academies d'agriculture de France", Académie national de médecine, Académie national de pharmacie, Institut de France Académie des Sciences, Académie of des Téchnologies, Académie Vétérinaire de France. http://www.Academie-Sciences.fr/activite/rapport/avis1012.pdf.

48 VBio (2012),"Rat study serious flaws", http://www.vbio.de/Informationen/alle_news/e17162?news_id=14723.

49 http://ec.europa.eu/food/food/biotechnology/index_en.htm.

50 See supra, note 1.