



*Committee for Independent Research and
Information on Genetic Engineering*

Report on NK 603 GM maize produced by Monsanto company

June 2007

Controversial effects on health reported after subchronic toxicity test: 90-day study feeding rats

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Last common ref. of the three authors: New Analysis of a Rat Feeding Study with a Genetically Modified Maize Reveals Signs of Hepatorenal Toxicity Arch. Environ. Contam. Toxicol. 52, 596–602 (2007)MON 863.

INTRODUCTION

Background information. NK 603 is a GM maize of the first generation, the first category of GMOs (the most important in the world, almost three-quarters of them) which were introduced onto the market. It is genetically modified to tolerate a herbicide. The first generation of GMOs in commercial use on open fields since 1995 either tolerate a pesticide in the first category (71% of GMOs - like Monsanto's RR soya or NK 603 maize - for instance tolerate primarily the Roundup herbicide) or produce a pesticide in the second category (generally, with artificial Bt toxins as in MON 810 or MON 863 maize, around one kg per ha; these different insecticides are produced in 18 % of GMOs). The second generation of GMOs (11% of total) developed as from 1998 do both:

they produce *and* tolerate a pesticide. Thus virtually all GMOs commercialised in agriculture have been designed to contain pesticides that they absorb and/or produce (all the remaining types make up less than 1%). The third and fourth generations are anticipated by actual experiments in fields, producing two insecticides and tolerating one or two herbicides.

NK 603 *description*. The genetic modification consisted of the chance insertion of an artificial genetic construction, called the transgene, by particle bombardment in immature cell maize genomes. The particles were covered by DNA fragments isolated from a plasmid; these fragments included two 5-enol pyruvyl shikimate 3-phosphate synthase (EPSPS) genes derived from the *Agrobacterium sp.* strain CP4, meant to induce resistance to glyphosate toxicity in plant cells. Normally, glyphosate inhibits the wild EPSPS endogenous enzyme from the plant, so that the normal plant treated with this total herbicide, which is widely used in the world, is not able to produce aminoacids essential for protein synthesis, and dies. The transgenes avoid this phenomenon, and the transgenic plants absorb the Roundup herbicide containing glyphosate, but are still able to have a normal protein synthesis. This fragment appears to be dominant and inherited in a Mendelian fashion. The transformed cells have then regenerated new, transformed plants, so-called GMOs. Everyone agrees that this may have created insertional mutagenesis effects that are not visible in compositional analysis; this kind of analysis by 'substantial equivalence' can by definition only be partial. From a reductionist point of view, the hypothesis made is that an artificial genetic modification by particle bombardment (or by an equivalent method) does not create more risk than unknown genetic effects possibly visible after classical hybridisation. This hypothesis has not been proven yet, but has been used to avoid labelling and long-term feeding studies with GMOs in North America. Thus, the side effects of pesticides residues on health are not studied over the long term, in contrast to what is done for some chemical pesticides. The toxicological tests last two years for pesticides, and are made on three species over 90 days.

In this context, the result of the confidential toxicity 90 day study with rats only is of the highest importance, because it is the most extensive material available so far for getting an idea of the potential effect of this GMO in mammals, or other unexpected effects of the genetic modification.

NK 603 is also designed to be used in combination with other GMOs, since several applications in the EU are related to hybrids with NK 603, and these even contain other GM characters, for instance producing different insecticides.

DOCUMENTS USED FOR THIS REPORT

For this report, we have compared and compiled four kinds of documents:

- 1) Background documents in the public domain for general and specific considerations (like EFSA or AFSSA reports)
- 2) Scientific peer-reviewed literature from various international journals. This literature is cited on www.ncbi.nlm.nih.gov/pubmed and mostly in *Ces OGM qui changent le monde*.
- 3) Files made available by Greenpeace and not covered by confidential agreements
- 4) Reports obtained via CRIIGEN (www.criigen.org) not covered by confidential agreements and communicated as such by the French government upon request; they are considered as public data. The government has given these to CRIIGEN upon the instruction of CADA (Commission of Access to Administrative Documents). These documents are written by Monsanto and different member states and are particularly concerned with relevant questions about the toxicity of NK 603.

REMARKS

All the scientific committees consulted agree with Monsanto that statistically significant differences were reported during the 90 day study on rats that had been controlled and treated (with GMOs) applying numerous parameters including blood composition and detoxification organs. Almost 70 (67) significant statistical differences were reported.

Significant effects in comparison to controls have also been noticed with other GMOs tolerant to Roundup, with at least four GMOs subjected to this kind of test resembling classical side-effects of pesticides in toxicology. But this has also been observed for MON 810 maize which is producing another insecticide (modified Cry1Ab): "For rats fed 33% MON 810 maize, a statistically significantly lower albumin/globulin count was observed compared with control and overall reference lines at study termination." (EFSA Journal 2004, 49, 1-25, page 15)

In fact, the data provided are taken from a 13-week feeding study in rats fed with Roundup Ready® corn (NK 603) preceded by a one-week baseline food consumption determination with PMI certified rodent diet # 5002.

The goal of the study (MSE-N 99091) was to compare the responses of rats fed diets containing a Roundup Ready® NK 603 corn to rats fed (1) diets containing the parental variety (non transgenic) corn and (2) a series of six diets containing grain from nontransgenic corn hybrids, designated reference controls. The study was conducted in Monsanto's Newsted laboratory; metabolism and safety evaluations were assessed in concordance with the standards of OECD (1997) good laboratory practice and WHLW good laboratory practice (GLP).

Male and female Sprague Dawley rats (six weeks of age, 20 rats/sex/group) were fed one of the following diets for 13 weeks: diets containing 11 or 33% event NK 603 corn, diets containing 11 or 33% (isogenic) parental control corn or diets containing 33% reference control corn (six commercial hybrids were tested). The diets which contained 11% test (NK603) or parent line were supplemented with 22% corn (nontransgenic commercial hybrid). PMI certified Rodent diet #5002 was administered during week 1 to establish the baseline food consumption for each animal.

Diet	Group	Material ID (% in diet)	Animals/ sex
Test NK603	M1/F1	NK603-L (11%)	20
Test NK603	M2/F2	NK603-H (33%)	20
Parent line	M3/F3	Parent-L (11%)	20
Parent line	M4/F4	Parent-H (33%)	20
Reference line	M5/F5	Crows 363 (33%)	20
Reference line	M6/F6	Pioneer 3394 (33%)	20
Reference line	M7/F7	Croplan Genetics 461 (33%)	20

Reference line	M8/F8	Campbells 6995 (33%)	20
Reference line	M9/F9	DK539 (33%)	20
Reference line	M10/F10	DK537 (33%)	20

Rats were assigned to groups on day 5 using randomisation that generated groups with no statistically significant differences in body weight. Rats were housed in individual cages, and diet and water were available as *ad libitum*.

- In each of the first two weeks of the study food consumption was measured daily on days 1, 2, 3 and over days 4-7. After week 2, food consumption was measured weekly.
- All animals were observed twice daily for morbidity and moribundity.
- Body weight was recorded at weekly intervals.
- After 5 and 14 weeks, blood and urine were collected from 10 animals from both sexes and each group for blood chemistry, hematology and qualitative and quantitative urine analysis.
- Coagulation parameters were determined at the terminal blood collections only.
- After 14 weeks, all animals were killed and necropsied. Specific tissues were collected and organs were weighed. Selected tissues were examined microscopically. There were two mortalities during the study. These were not considered as being related to the treatment.

In total, some 1050 comparisons were made and approximately 53 of these were anticipated as being significant by chance alone at the 5% significance level.

Almost 70 (67) statistically significant differences have been observed and reported by the Monsanto's statistical analysis - 12 for hematology parameters (hematocrit, platelets, neutrophils, lymphocytes, monocytes, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration), 18 for clinical chemistry parameters (albumin, blood urea nitrogen, creatinine, phosphorus, sodium, chloride, alkaline phosphatase, calcium, potassium), nine for urine chemistry parameters (creatinine, phosphorus, potassium, creatinine clearance, pH, calcium), six for the organ weights (brain, heart, liver), 14 for body weights and body weight changes, and eight for food consumption.

Nevertheless the final report concludes that "...rats fed corn grain containing event NK 603 corn responded similarly to rats fed parental and reference control grain.." and that "... Roundup Ready NK 603 corn is equivalent to its parent control line and nontransgenic commercial corn varieties..."

The reason for this conclusion comes from the observation that the number of significant observed differences is of the same order as the number expected by chance. Therefore, the statistical analysis concludes that those differences occur randomly, are not relevant and can not be considered biologically significant. But this is not final proof that the significant effects are not related, nor that they are not important for mammalian health. Further studies have to be conducted. There are a number of open questions and indications that Monsanto's conclusion is premature and the data have to undergo further investigation.

The few remarks which follow do not constitute, in any manner, a statistical analysis of the data from NK 603, but they point to elements that show the importance, on the one hand, of carrying out this statistical analysis in a serious and independent way and, on the other hand, of performing further testings or repeating the 90 days study.

Remark 1: It is very surprising that the experimental design was elaborated and performed at the MSE-N laboratory, a Monsanto company, and that the statistical analysis of the data was carried out by Monsanto's statistics centre. This is likely to seriously impair the independence of the expertise involved.

Remark 2: There are two kinds of possible effects in the consumption of NK603. NK603 corn contains a new genetic construction in its genome, and it was sprayed with glyphosate at commercial rates of application and thus may contain toxic residues of Roundup. The six reference groups have not been chosen in the right way for investigating these two groups of possible risks.

Remark 3: It is important to run further investigations, especially on the groups 1 to 4, the food for which includes 11% and 33% NK603 maize and its parental variety (isogenic, not genetically modified), using the method of a two-way ANOVA with specific focus on interactions (transgenic maize with glyphosate/nontransgenic and factor dose 11% / 33%).

Remark 4: The analysis of the rats' weights must be improved by a study and comparison of the curves of growth, and their correlations with the time series of consumption and the characteristics of the organs at the time animals were killed.

Remark 5: In the protocol there is a relatively high number of measurements of quantitative and qualitative variables for each rat and the distinctive groups. Therefore the statistical analysis used in these experiments should have been multidimensional from the beginning. In these circumstances it is very surprising that Monsanto's statisticians did not use standard multivariate methods like principal component analysis (PCA), Data Mining, Manova. Instead, most of the data are analysed with a simple Analysis Of Variance (ANOVA) with only one factor, week after week, character after character, which means the focus of the statistical analyses was directed rather to the margins and not on the joint data. Under these conditions important parts of the information, particularly possible correlations and interactions between the biological parameters and the organs, are likely to become lost, with a risk of neglecting effects and differences which could be biologically significant.

Remark 6: The statistical analysis must be completed with data being interpreted by biologists, toxicologists and physicians (pathologists) in order to correlate the statistically significant differences and the possible development of signs of toxicity, clinical symptoms or pathologies.

For all these reasons a deeper statistical analysis of the data should be performed, especially to exclude any risks to human health.

DISCUSSION

Interpretations of above data. Most of these significant differences were judged "not biologically meaningful" by Monsanto and then, after important debates and meetings, by the majority of experts in several scientific committees. The results were considered important but the interpretation of the data gave reasons for disagreements. One reason for the judgement of the majority of experts might be that so far only a very few number of toxicologists have been involved in the final interpretation of the data, and to our knowledge none of them had access to the histological slides of the organs except for those selected by Monsanto.

Anyway, the main arguments in discussions supporting Monsanto's view that there are no toxicity signs were:

1/ The data of the additional reference groups of those rats fed with normal maize [which was not similar to the original isogenic maize used for the genetic modification] were used to explain the observed effects as a matter of normal biological variance. But normally the data from those control groups fed with a very closely related (isogenic) maize are seen as relevant because under these conditions the difference in the diet (and its related biological effects) can be considered to be caused by the transgene, its protein expression and its effects alone, like herbicide-related residues. This is a general practice with GMO tests. In the actual experiments the total reference group was at least three times bigger than the GMO-treated group (and finally in some instances even the historical data of the laboratory conducting the experiment served as references for some effects observed in the experiment!).

2/ Where there were some significant effects, the differential effects between males and females were used to say that the problem was probably not linked to the GMO.

3/ With some significant effects, observations made only during some weeks of the experiment served as an argument to eliminate their biological significance.

4/ With some significant effects, the absence of correlations with the dose ingested by the rats was a reason for avoiding linking them to the GMO.

But, by contrast, another interpretation of the data presented based on more complex (but also more adequate) statistical methods and more detailed interpretation of the observed biological effects seems to be necessary:

1/ The statistical analysis may have encountered problems in the choice of methodology or unexpected bias and should be made again. CRIIGEN proposed this after initial examination of all the crude data. The improper or poor statistical analysis has been already discussed; it has been admitted in other similar cases that such an analysis is lacking. (Ref. New Analysis of a Rat Feeding Study with a Genetically Modified Maize Reveals Signs of Hepatorenal Toxicity, Arch. Environ. Contam. Toxicol. 52, 596–602 (2007).)

2/ The differential effects of a treatment by a toxic compound on males and females are observed quite often; this may be due to enzymatic and hormonal differences in detoxification between the two sexes.

3/ The transient effects after chemical or biological intoxications are also numerous and do not mean that the compound is safe in the long term.

4/ Dose-dependent effects are not the only ones to be taken in consideration in toxicology. For instance, most endocrine effects are not with certainty directly proportional to the dose, but may present biphasic or feedback effects, and they also depend on the time of exposure. Moreover, two doses are insufficient to measure a dose-related effect.

CONCLUSION

It can be concluded that no independent study of toxicity has been made other than the experiments directed and interpreted by the Monsanto company. In addition, the interpretations of data may be controversial. There was no open access to the organs from treated rats and slides of these organs.

The confidentiality of raw data material as claimed by Monsanto has no scientific or legal basis; all scientific data have to be published or made transparent as they are in market applications to the EU or its member states, as is done for public research if the GMO is meant to be used for food or feed in the EU. Also directive CEE/2001/18 indicates that risk assessment for health and the environment should be public for GMOs.

Whatever the results are, in such a controversial case the minimum expectation could be, as in public research, for the experiment to be repeated if no final conclusion can be drawn from the current data. CRIIGEN proposes to conduct new experiments - longer and on two generations of

rats - and is asking for financial support for this project, which would be conducted applying OCDE standards.

The closest comparison to testing GMOs for safety that might be made is presented by pesticides, since this GMO has been genetically modified in order to tolerate a pesticide. European legislation on pesticides has for a long time been regulated by the directive CEE/91/414 and its successive amended versions. Where the toxicity study of pesticides in food and feed for humans and other mammals is concerned, this legislation lays down that three-month tests should be done for three species (generally rat, mouse and dog), and that pesticides are administered in food for one year to one species (generally a dog), and for two years to another (generally a rat, and approximately corresponding to its lifespan). There is no scientific reason for not carrying out experiments of this kind for the current GMOs. But these scientifically derived preconditions for testing GMOs before allowing them to go on the market might be seen as an ethical hurdle for market authorization if no adequate benefits can be expected from such products when compared to the disadvantages in making tests on animals.

The in vivo tests should be conducted as a final safeguard in testing unknown products that do not present negative in vitro effects. However, the use of specific in vitro tests should be encouraged by EU authorities before animal testings are performed; there is very large room for further improvements in GMO authorisation procedure.

In the case of NK 603 maize, it should be noted that the 90-day toxicology study appears to be the best published to date and the longest that has been performed with mammals. It shows significant effects in comparison to control laboratory animals, and in some instances even in comparison to the so-called very large "reference group". In all instances, it is recommended that:

- 1) The statistical analysis should be repeated by independent experts and the crude data put on a website for the scientific community.
- 2) The experiment should be repeated if the significant effects, as compared to the proper control group, are confirmed.
- 3) Other experiments with rats for one and two years, and with two other species of mammals, should be conducted in order to study potential adverse effects of the genetic modification, to know if these are linked to the Roundup residues present in the maize, the genetic modification itself or other unintended effects. GMOs should not be exempted from pesticide evaluation if they contain pesticides or specific pesticide metabolites. NK603 should obviously be tested for glyphosate residues and other adjuvant Roundup residues. Some of these have been shown to be toxic for human cells (Richard et al., 2005, Environ. Health Perspect. 113, 6, 716-720 & Benachour et al., 2007, Arch. Environ. Contam. Toxicol. 53, 126-133).

In the absence of such results, consent for maize to be released into the environment, for food, feed or cultures, may present a serious risk to human and animal health and releasing it should be forbidden.

One should also underline that there is today no legal obligation on companies concerning the exact basic number or length of studies they have to make on mammals eating GMOs. This lack of precision (e.g. the Entransfood project) makes matters difficult for state authorities and companies. It also gives consumers reason to put in question the safety of GMO-derived products. Therefore a mandatory protocol for experimental testings as is used in pesticide testing (combined with a clearing procedure for ethical questions related for instance to animal experiments) could help to settle public and scientific controversies and provide more evidence about the quality and the safety of these products.
