Report on MON 863 GM maize produced by MONSANTO Company
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Controversial effects on health reported after subchronic toxicity test : a confidential rat 90 day feeding study

By Pr. Gilles-Eric SERALINI, University of Caen, France and President of the Scientific Council of the Committee for Independant Research and Information on Genetic Engineering (CRIIGEN).
Professor SERALINI is member of two commissions for GMOs evaluation before and after commercial release (commissions of the french Ministry of Agriculture and Ministry of Ecology) since 1998, and was expert for the European authorities on the first panel in the WTO conflict with the United States, concerning the GMO moratorium. He develops research on the effects of pesticides on health. He has written several books on GMOs in french, the last one is « Ces OGM qui changent le monde » (Ed. Flammarion).

INTRODUCTION

Background information. MON 863 is a GM maize from the first generation, second category of GMOs ; i.e. genetically modified to produce a pesticide. The first generation of GMOs commercialized in open fields since 1995 either tolerate a pesticide for the first category (72% of GMOs tolerate for instance mainly the herbicide Roundup, like NK603 maize from Monsanto) or produce a pesticide for the second category (generally around a kg/ha, like artificial Bt toxins in MON810 or MON863 maize ; these different insecticides are produced in 20% of GMOs). The second generation of GMOs (8% of total) developed from 1998 make both : producing *and* tolerating a pesticide. Then virtually all GMOs commercialized in agriculture have been designed to contain pesticides that they absorb and / or produce (all the remaining characters are less than 1%). The third and fourth generations are anticipated from the actual experiments in fields to produce two insecticides and to tolerate one or two herbicides.

MON 863 *description.* The genetic modification has inserted an artificial genetic construction, called the transgene, by particle bombardment by chance in the maize genome from immature cells. These cells have then regenerated new transformed plants, so called GMOs. Everyone agrees that this may have created insertional mutagenesis effects that are not visible by the compositional analysis ; this kind of analysis by « substantial equivalence » can by definition only be partial. From a reductionist point of view, the hypothesis taken 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 hybridization. This hypothesis has not been demonstrated yet, but has been used to avoid labelling and long-term feeding studies with GMOs in north america.
In our precise file, the genetic modification has been performed and reaches three separate goals :

1) to produce a variant of an artificial insecticide called Cry3Bb1 by the maize plant (49-96.5 µg/g) throughout its development and in all organs (adapted 35S promoter is used in the genetic construction). The toxin is measured in the grains by Monsanto. This toxin is directed against coleopteran insects like Diabrotica. Diabrotica is from a very dangerous family of insects for a wide range of crops and was absent from the european countries untill the late 1990's, forbidden even in laboratories (*Diabrotica virgifera virgifera*) because it is very difficult to eliminate it with known chemical insecticides. It appears that it has been introduced probably during the Balkan's war from America ; and since then it reaches western european countries like Italy and France (around 2000), probably by planes (around military airports). Monsanto seems to have anticipated this problem since the company developed a few years before transgenic maize against to try to fight this insect in the future, in trials in particular in France ; this step being necessary some years before commercialization

in the country. The molecular mechanism of action of the toxin is not precisely known, nor is identified the receptor binding the toxin within the insect gut. The specificity of action is generally hypothesized ; but no proof has been published on the action of this toxin on human cells and a controversy exists at this level. Monsanto is not able to produce toxicity tests with the toxin extracted from the maize and put into contact with human digestive epithelia. It appears that following theoretical considerations, and preliminary data from acute toxicity experiments during a few days in a very little number of rodents, the toxin has been exempted from serious toxicity analysis. In this context, the result of the confidential toxicity 90 day study with rats is of highest importance, because it is the best that one can have to get an idea of the toxin activity in mammals, or other unexpected effects of the genetic modification.

2) To facilitate economically the maize selection, Monsanto has used and maintained within the GM plants an antibiotic marker gene called NPTII (neomycin phosphotransferase II). The latter produces into the vegetal cells a protein inducing resistance towards at least kanamycin, a well known antibiotic. This is also a sign of the first generation of GMOs which have been made rapidly with low consideration of the following problem. Antibiotic resistance is recognized to be a major health problem in numerous countries, developed because of the growing development in the environment and bodies of antibiotic resistance genes. This is a phenomenon amplified by the common use of antibiotics according to the scientific community, which agrees to limitate their use nowadays. In this context, it could appear very strange to promote a food containing an antibiotic resistance, overall since Monsanto has already developed transgenic maizes without this kind of marker genes. This is true even if the company says that the antibiotic resistance has little chance to spread out from this agriculture, and that this will have if any very little effect on human and animal health. This belief is not supported by well-designed experiments to prove it. This could shed a very big trouble in citizens' mind on the real goals of this company on health protection ; and this is not good for the development of biotechnologies in general, that have been highly promoted by member states policy, including by heavy financial supports.

3) MON 863 is also designed to be a transgenic father for other GMOs, since several applications may concern hybrids with MON863, even containing other GM characters, like MON 863 x MON 810 producing two different insecticides (next generation). Although MON 863 is giving its Cry3Bb1 toxin to MON 863 x MON 810 plants in this instance, both plants are genetically different and are not a priori directly comparable for their toxicity. Combined effects of both insecticides are not excluded.

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 non covered by confidential agreements
4) Reports obtained via CRIIGEN (*www.criigen.org*) non covered by confidential agreements and communicated as such by the french government after request ; they are considered as public data. The government has given that to CRIIGEN after order from CADA (Commission of Access to Administrative Documents). These documents are written by Monsanto and different State Members asking relevant questions about toxicity of MON 863 in particular.

RESULTS

All the scientific committees consulted agree with Monsanto that statistical significative differences (summarized below) have been reported during the 90 day study between control and treated rats (with GMOs) on numerous parameters including blood composition and detoxification organs such as kidneys. EFSA indicates at this level : « Some differences were observed in haematological parameters, including total white blood cell, lymphocyte and basophil counts » ; « At study termination, statistically significant differences were observed for reticulocyte counts between the female animals fed 33% MON 863 and those fed the control and reference lines » ; « Individual kidney weights of male ratd fed with the 33% MON 863 diet were statistically significantly lower compared to those of animals on control diets » ; « a statistically significant lower incidence of mineralized kidney tubules was noted for rats fed 33% MON 863 maize compared to those fed the control maize ».

Significant effects in comparison to controls are also noticed with other GMOs tolerant to Roundup, and in total with at least 4 GMOs for which this kind of tests has been done, resembling classical side-effects of pesticides in toxicology. But this has also been observed for MON 810 maize producing another insecticide : « 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 ».

On the other hand, public CGB discussions report inflammation and regeneration abnormalities in male kidneys fed with MON 863, significant increase of glycemia in treated females. Scientific committees in Austria, Italy, France, Spain, Sweden, and The Netherlands in particular have given questions to Monsanto on toxicity and allergenicity of this maize or MON 810, or both, or MON 863 x MON 810 after the transmission of the Company data, even if the time to evaluate the documents was very short.

DISCUSSION

Interpretations of above data. Most of these significant differences were judged as « not biologically meaningfull » by Monsanto and consecutively by several scientific committes cited above at majority, after important debates and meetings. The results were considered as relevant but their interpretation may be the cause of disagreements. Some of the final votes were made with a very few number of toxicologists, and none of them had access to our knowledge to the histological slides of the organs except the ones decided by Monsanto Company.

Anyway, the main arguments in discussion in favour of the absence of toxic effects were :
1/ The comparison with reference groups of rats that did not eat the same line of maize than the maize that was genetically modified. By contrast, the control group was fed with a maize genetically very close to the treated group with the GMO, the difference in the diet was considered in this case to be the transgene, its protein expression and its consequences alone. This is a general practice with GMO tests. The total reference group was also at least 6 times bigger than the GMO treated group (in some instances the historical data of the laboratory conducting the experiment served also as references in some files).
2/ For some significant effects, the differential effects between males and females served to say that the problem was probably not linked to the GMO.
3/ For some significant effects, their observations only during some weeks of the experiment served to eliminate those from biological significance.
4/ For some significant effects, the absence of correlation with the dose ingested by the rats was a cause to avoid to link them to the GMO.

By contrast, other international experts, after consultation may consider that :

1/ The statistical analysis may have encountered problems in the choice of methodology or unexpected bias and should be done again. This is proposed by CRIIGEN after communication of all crude data. The improper or poor statistical analysis has been admitted in some cases.
2/ The differential effects of a treatment by a toxic coumpound on males and females is observed quite often, this may be due to enzymatic and hormonal differences between the two sexes in regard to detoxification.
3/ The transient effects after chemical or biological intoxications are also numerous and do not mean that the coumpound is safe on a long-term.
4/ The dose-dependant effects are not the only ones to be taken in consideration in toxicology. For instance, most of endocrine effects are not for sure directly proportional to the dose, but may present biphasic or feedback effects, and also depend on the time of administration.

CONCLUSION

It can be concluded that no independant study of toxicity has been made besides the experiments directed and interpreted by 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. There was never new experiments after discussions, but only new analysis and interpretations of the same MON 863 data by experts designed by Monsanto. Moreover and for instance, for all GMOs untill recent years, the so called independant external expert paid by the french government to be referee for CGB was, according a written rule, chosen during numerous years by the Company in the last round of propositions. Even if that is not always the case now, it should be checked if this kind of practice is followed by other state members or EU. All these practices avoid a contradictory expertise similar to judiciary processes, but this could be organized easily. The secret on confidential raw data claimed by Monsanto has no scientific basis ; all scientific data have to be published or transparent are they are in the commercial request files to the state members, like it is done for public research, if the GMO is for public feeding. The directive CEE/2001/18 indicates that the risk assessment on health and environment should be public for GMOs.

Whatever the results are, in such a controversial case, the minimum could be, like in public research, to repeat the experiment since no clear conclusion can be drawn from these data. CRIIGEN proposes to conduct new experiments, also longer and on two generations of rats, and is asking for financial support for this project, which is ready to go with OCDE standards.

If we compare GMOs with other products tested for their safety, the closest example possible is for pesticides, since this MON 863 GMO has been genetically modified in order to produce a pesticide. The european legislation concerning pesticides has been for a long time directed by the directive CEE/91/414, and its successive adaptations. This legislation precises that, concerning the toxicity study of pesticides in food and feed for humans and other mammals, three month tests should be done for three species (generally rat, mouse, and dog), and that pesticides are given in food during one year to one species (generally dog) and during two years on another one (generally rat, this approximately corresponds to its life span). There is no scientific reason to avoid these kind of experiments for actual GMOs.

The in vivo tests are the final security that should be undertaken to test unknown products that do not present in vitro negative effects. However, specific in vitro tests should be stimulated before, and one can note that there is very large room for still improvements in GMO files, i.e. more tests with the Bt artificial Cry3Bb1 toxin extracted from the maize and incubated with human cells in this case.

In the case of MON 863 maize, it should be noted that the 90 day toxicology study appears to be the best one and the longest one that has been performed with mammals. It shows significant effects in

comparison to control laboratory animals, and in some instances in comparison to the so called very large "reference group", the existence of which may be questioned. In all instances, it is recommended that :
1) The statistical analysis should be repeated with independant experts and the tests put on a website for the scientific community
2) The experiment should be repeated if the significant effects are confirmed, in comparison with the proper control group
3) Other experiments with rats during one and two years, and also 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 Cry3Bb1 toxin or not, like it is regularly performed for other pesticides. GMOs should not be exempted from pesticide evaluation if they contain pesticides or specific pesticide metabolites. It is the case obviously for MON 863.
4) In vitro studies should be performed with Cry3Bb1 extracted from maize and various mammalian cells including human digestive epithelia and hepatocytes
In the absence of such results, the agreement for maize release into the environment, for food, feed or cultures, may present a serious risk for human and animal health and the release should be forbidden.

One should also underline that today no legal obligation is given to companies concerning the exact basic number of studies they have to accomplish on mammals eating GMOs and their length. This lack of precision (Entransfood project) is difficult for public authorities and companies. For the public, it could appear very normal to give GMOs during 2 years to rats before giving them to the entire population during their entire life, including babies and elderly or sick people. To standardize the GMOs tests in Europe on three mammalian species, from 3 months to 2 years, could finally help companies to reach homogenized standards and to commercialize high quality food and feed. Biotechnology will be more easily accepted in such conditions.

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