

## New Analysis of a Rat Feeding Study with a Genetically Modified Maize Reveals Signs of Hepatorenal Toxicity

Gilles-Eric Séralini,<sup>1,2</sup> Dominique Cellier,<sup>1,3</sup> Joël Spiroux de Vendomois<sup>1</sup>

<sup>1</sup> Committee for Independent Information and Research on Genetic Engineering CRIIGEN, Paris, France

<sup>2</sup> Laboratory of Biochemistry, Institute of Biology, University of Caen, Caen, France

<sup>3</sup> Laboratory LITIS, University of Rouen, Mont-Saint-Aignan, France

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**Abstract.** Health risk assessment of genetically modified organisms (GMOs) cultivated for food or feed is under debate throughout the world, and very little data have been published on mid- or long-term toxicological studies with mammals. One of these studies performed under the responsibility of Monsanto Company with a transgenic corn MON863 has been subjected to questions from regulatory reviewers in Europe, where it was finally approved in 2005. This necessitated a new assessment of kidney pathological findings, and the results remained controversial. An Appeal Court action in Germany (Münster) allowed public access in June 2005 to all the crude data from this 90-day rat-feeding study. We independently re-analyzed these data. Appropriate statistics were added, such as a multivariate analysis of the growth curves, and for biochemical parameters comparisons between GMO-treated rats and the controls fed with an equivalent normal diet, and separately with six reference diets with different compositions. We observed that after the consumption of MON863, rats showed slight but dose-related significant variations in growth for both sexes, resulting in 3.3% decrease in weight for males and 3.7% increase for females. Chemistry measurements reveal signs of hepatorenal toxicity, marked also by differential sensitivities in males and females. Triglycerides increased by 24–40% in females (either at week 14, dose 11% or at week 5, dose 33%, respectively); urine phosphorus and sodium excretions diminished in males by 31–35% (week 14, dose 33%) for the most important results significantly linked to the treatment in comparison to seven diets tested. Longer experiments are essential in order to indicate the real nature and extent of the possible pathology; with the present data it cannot be concluded that GM corn MON863 is a safe product.

numerous blood and organs parameters measured (Domingo 2000; Meningaud *et al.* 2001) and only one study with the MON 863 maize in such conditions. It has been performed under the responsibility of Monsanto Company and was recently published after the authorities' assessment (Hammond *et al.* 2006). The crude data at first kept confidential were subjected to questions from regulatory reviewers in Europe, where it was finally approved in 2005. This necessitated, in particular, a new assessment of kidney pathological findings, and because the study was claimed afterwards to provide an assurance of safety (Hammond *et al.* 2006), we independently re-analyzed these data here obtained after a Court action. The whole approval was based on the statement that all the significant differences were not biologically meaningful. To assess this hypothesis, we wanted to link the statistical differences per organ and to apply new methods of analysis. This transgenic maize was modified to produce in its cells a new artificial insecticidal and modified toxin Cry3Bb1 (49–97 µg/g) that was exempted from subchronic toxicity *in vivo* studies (Hammond *et al.* 2006), and its mechanism of action is not known in mammals, because it was not tested, and the target receptor has not been characterized precisely in insects.

Most, if not all, of the commercialized genetically modified organisms (GMOs) in open fields contain pesticide residues that they tolerate and/or produce (Clive 2006). Regulatory rules do not require 3-month tests with three mammalian species, then with a mammal for 1 year and yet another for 2 years, such as those employed for the testing of pesticides or drugs. This is why it appears crucial to analyze carefully the longest toxicity tests available only in one mammalian species, where numerous parameters have been measured for 400 rats, according to Organisation for Economic Co-operation and Development (OECD) standards during only 90 days. Other independent studies over 8 months with mice fed a GM Roundup tolerant soy were very detailed but only at an ultrastructural level, and showed nuclear transcription abnormalities in hepatocytes during the feeding (Malatesta *et al.* 2002), in pancreas (Malatesta *et al.* 2003), and testes (Vecchio *et al.* 2004), and hypothesized that these changes might be due to Roundup herbicide (Monsanto) toxic effects, similar to those observed on mammalian cells (Richard *et al.* 2005), but

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Very little data have been published on mid- or long-term feeding studies with genetically modified plants, approved and commercialized, in equilibrated diets, given to mammals, with

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Correspondence to: Gilles-Eric Séralini; email: criigen@unicaen.fr

the parameters measured in these longest toxicity tests published on GMOs did not concern almost all organs and blood and urine chemistry, as in the present experiment.

## Materials and Methods

### *Biological Context: The In Vivo Protocol of Monsanto*

All OECD standards were claimed to be followed by the Monsanto Company: individual cages, animal randomly distributed in each group after a 1-week stabilization period, standard and validated measurement methods, and so on. This feeding study served to authorize the MON863 maize by the European and American authorities. It included young adult Sprague-Dawley-derived rats (CrI:CD<sup>®</sup>(SD)IGS BR, Charles River Laboratories, NY), approximately 6 weeks old separated in 10 groups of 20 males and 10 of 20 females analyzed in details (organ weights and histology), but the biochemical parameters were measured only for half of these at weeks 5 and 14. For each sex, two groups were fed with GMOs, one with 11% and the second with 33% of MON 863 in the equilibrated diet, and two with the closest control line and regimen, grown in the same location (Hawaii), called control herein, indicated to be substantially equivalent (Hammond *et al.* 2006), in similar proportions. The closest control plant possible will then be the equivalent isogenic or parental nontransformed line, grown in similar conditions. In this article, the control is called the LH82 × A634 line. The six other groups were given diets without GMOs but that did not have the same final chemical composition, even if these diets also met PMI specifications for Certified 5002 Rodent Diet. They contained 33% of conventional different maize lines (MON 847 Repl, Asgrow RX-770, LH235 × LH185, LH200 × LH172, B73Ht × LH82, Burrus BX-86). These were not grown in the same locations (Illinois or other places in Hawaii), and were not demonstrated to be substantially equivalent to the GMO and control diet, but were supposed to mimic the variability of regular reference regimens, called reference herein, and other details have been described (Hammond *et al.* 2006).

The genetic modification in the maize tested here was inserted by chance by particle bombardment in the plant genome of immature cells. This may cause insertional mutagenesis effects, which may not be directly visible by compositional analysis; the latter can then be only partially compared for a nonexhaustive list of substances to conventionally bred lines, for instance, to test “substantial equivalence.” The genetic construction itself comprises a transgene with an ubiquitous adapted 35S promoter encoding a modified toxin directed against the coleopteran insect *Diabrotica*. This dangerous parasite was probably introduced several times by airplane in Europe from the late 1990s (Miller *et al.* 2005). The problem apparently has been anticipated by the first trials of MON 863 or similar GMOs in Europe. This maize also contains a neomycin phosphotransferase II marker gene, coding for antibiotic resistance, to facilitate the selection of the transformed plants.

### *Statistical Methods*

The present feeding experiment was designed and statistically assessed by Monsanto Company (St. Louis, MO), but animals were analyzed by Covance Laboratory (Vienna, VA). We first repeated the same statistical analysis as that of Monsanto to verify descriptive statistics (sample size, means, standard deviations) and one-way analysis of variance (ANOVA) by sex and by variable. For that, the normality of the residues was tested using the Shapiro test and the

homoscedasticity (homogeneity of the variances) using the Bartlett test. In the case where the Shapiro and Bartlett tests were nonsignificant ( $*p > 0.05$  and  $**p > 0.01$ , respectively) we performed an ANOVA, and in the case of heteroscedasticity the approximate Welch method was used. In the case where the Shapiro test was significant, we performed the Kruskal-Wallis rank sum test.

In addition, we undertook a multivariate analysis of the growth curves and the consumption of the rats. For the weight growth curve of the rats, after linear regression, the weekly relative increase rate can be considered proportional to the logarithm of the weight, and thus we used a Gompertz model (Ratkowsky 1990; Huet *et al.* 2004),  $Y = a \cdot \exp(-\exp(-b(X-c)))$ . The parameter  $a$  represents the top of the curve,  $b$  is related to the growth rate, and  $c$  is a position parameter with the X axis. These parameters were estimated by nonlinear regression. In order to see whether the growth curves are significantly different, we compared the models by testing the null hypothesis (which would give the same curves with identical parameters for both groups) against the alternative (different curves). For that, we used the  $F$  test to compare the sum of square errors under the two hypotheses. The Akaike's Information Criteria (AIC, Akaike 1974) was also used to evaluate the probability of differences.

We then analyzed the GMO effects for each sex and each diet by pairwise comparisons of the parameters of GMO-fed rats to the control groups and after to the reference groups. In order to select the appropriate two-tailed comparison test (Crawley 2005), we again studied first normality (Shapiro test) and variance equality ( $F$  test). According to the results, we performed the adapted test, i.e., an unpaired  $t$  test, a Welch corrected  $t$  test or a Mann-Whitney test (which is generally more appropriate with a sample size of 10).

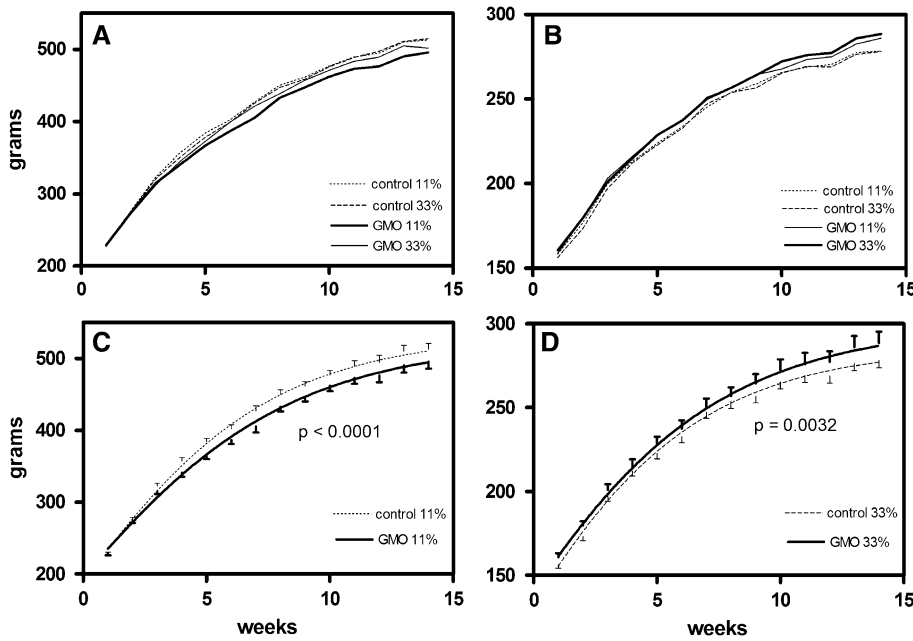
We used the R language (Crawley 1995) version 2.2.1 for statistical computations (Comprehensive R Archive Network, CRAN - <http://cran.r-project.org>), except for the weight growth curves statistical study, for which nonlinear regressions were performed using GraphPad Prism (version 4.02 for Windows, GraphPad Software, San Diego, CA, [www.graphpad.com](http://www.graphpad.com)).

## Results

We first checked all the crude data, and we noticed a concordance for descriptive statistics (sample size, means, standard deviations) and one-way ANOVA by sex and by variable between our calculated values and those published by Hammond *et al.* (2006) from Monsanto Company.

### *Body Weights*

Our study consisted of a multivariate analysis of the growth curve and the consumption of the rats for the four groups receiving GMOs or equivalent diets. If the animal consumption was not noticeably changed, it appeared for the growth curves that the variations for the two controls for each sex are superimposed, whereas the GMO feeding trials provoked different growths (Fig. 1). The 11% GMO groups were always under the 33% groups for both sexes. All the males are growing less than the controls from week 2, and all the females more. This sex- and dose-related effect resulted in the fact that the growth variations of the 11% GMO males are highly statistically lower than their controls, and 33%-GM fed females higher (Table 1). All  $p$  values of different groups versus controls are  $<0.01$ . This results in 3.3% decrease in weight for males and 3.7% increase for females.



**Fig. 1.** Body weight growth for males (A, C) and females (B, D) over a period of 14 weeks. The experimental (A, B) and corresponding theoretical curves according to Gompertz models (C, D) are presented. The most important effects in each sex are in bold lines and statistically different from controls (see Materials and Methods)

**Table 1.** Statistical differences between weight curves

a			
Gompertz models for males			
Par.	Control 11%	GMO 11%	One model
a	533.6	524.6	528.8
b	0.2240	0.2011	0.2126
c	0.1251	-0.0939	0.0185
Gompertz models for females			
	Control 33%	GMO 33%	One model
a	286.1	300.1	292.9
b	0.2272	0.2016	0.2142
c	-1.185	-1.376	-1.282
b			
Sex	F test		AIC
Males	<i>p</i> < 0.0001 <i>F</i> = 11.73		Prob. > 99.99% Diff. 28.34
Females	<i>P</i> = 0.0032 <i>F</i> = 4.66		Prob. = 98.04% Diff. 7.83

The parameter estimates for Gompertz models have been calculated (a) for parameters (Par.) a, b and c and tested for statistical differences (b, F test column 2) with p values and the F ratio. The Akaike's Information Criteria (AIC) and the probabilities (Prob.) for differences (Diff.) in curves are precised (b, column 3).

*Other Parameters*

We then studied first the GMO effects in comparison to the isogenic, nontransgenic, equivalent maize (control) in Table 2, then the effects of different nonequivalent maize compositions on rat physiology (six different reference groups versus controls). Finally, we studied the GMO effects versus all different diets (double frame, Table 2). In total, 58 biochemical parameters reflecting most physiological functions were measured two times (week 5 and 14), in particular through serum and urine chemistry, and hematology. Organ weights

and relative ratios were added. We thus performed 494 comparisons: 40 differences (8%) were statistically significant (\**p* < 0.05); 25 would have been expected under the global null hypothesis of no differences between GMO and control diet effects. Among the 40 significant differences, we retained only the 33 with a relatively  $\geq \pm 5\%$  difference to the mean; this most probably also excluded potential incidental differences, if any. Table 2 summarizes only the list of significantly disturbed parameters at least for one sex or one treatment, and also shows the percentage of variations of the means. The same Table 2 is obtained if we systematically use the

**Table 2.** Differences between GMO-fed rats and controls

	Week	m 11%	m 33%	f 11%	f 33%
<b>Liver parameters</b>					
Albumin/globulin ratio	5	11*	-3	-9	4
Albumin/globulin ratio	14	6	-2	-18**	7
Albumin	5	-3	-2	-2	5*
Albumin	14	-2	3	-6*	5
Globulin	5	-12*	2	9*	1
Globulin	14	-8	7	15*	-2
Alanine aminotransferase	14	-30*	-8	37	4
Total protein	14	-5*	5*	1	3
Triglycerides	5	22	-2	-11	40**
Triglycerides	14	15	-1	24*	6
Liver weight	14	-1	-2	7**	6
Liver/brain ratio	14	-1	-3	6*	4
<b>Kidney parameters</b>					
Creatinin	14	-7	13*	13*	-2
Urine sodium	14	-23	-25*	11	-26
Urine sodium excretion	14	3	-35*	35	-24
Urine chloride excretion	5	35	3	50*	67*
Urine potassium	5	35*	-20	-3	-13
Urine phosphorus	5	3	-35*	24	-15
Urine phosphorus	14	-34	-31*	12	-8
Urea nitrogen	14	-8	4	17*	-1
Kidney weight	14	-3	-7*	3	2
Kidney/brain ratio	14	-3	-7*	1	1
Kidney % body weight	14	-1	-5*	-1	-1
<b>Pancreas</b>					
Glucose	14	-4	9	9*	10**
<b>Bone marrow</b>					
Neutrophils	5	5	22*	-14	3
Eosniophils	14	32	54*	20	0
Reticulocytes	14	15	-17	-35	-52*
Reticulocytes % RBC	14	16	-16	-36	-55*

Study of the GMO effects indicated by mean differences (%) for each parameter with the corresponding control group per sex and per dose. The significant differences *versus* controls (\* $p < 0.05$ , \*\* $p < 0.01$ ), for all the parameters measured in the subchronic feeding tests, are presented. The parameters were grouped by organs according to the sites of synthesis or classical indicators of dysfunction. They were indicated for all groups only if they showed at least for one sex or one diet a significant and relatively  $\geq \pm 5\%$  difference to the mean. The animals were male (m) or female (f) young adult rats fed during 5 or 14 weeks with GMO (MON 863, 11 or 33% in the diet) and compared with controls fed with a “substantially equivalent” isogenic maize line (LH82  $\times$  A634) grown in the same location (Hawai). The parameters were measured for 10 rats, except for the organ weights (20 rats), obtained only at the end of the experiment. In single-boxed numbers, we indicate the statistical differences between GMO-fed rats and controls, which are not found between the mean of the six reference groups and controls. A difference between reference and control groups could indicate an effect of the diet per se. In double-boxed numbers, among the effects due to the GMO, are indicated the statistical differences between the GMO groups and the mean of the six reference groups (which have not even eaten the same composition as the control and the GMO treated groups).

Mann-Whitney test for all the biological parameters, except for albumin-14-f11%, urine phosphorus-5-m33%, and urea nitrogen-14-f11%; the  $p$  values in this case are comprised between 6.3% and 10.6%; these were not considered below. Table 3 corresponds to physiological values of the significantly disturbed parameters in GMO-fed rats in comparison to their corresponding controls. It emphasizes the impressive quantity of abnormalities.

Table 2 indicates that GMO-linked variations in comparison to controls were concentrated mostly on five male and nine female liver parameters, and nine and four kidney parameters

for males and females, respectively, on all organs studied. We then measured the significant variations between the six reference groups and controls (isogenic to GMO), which allowed us to study the potential effects of the diet composition alone. The parameters that were also disturbed in this case were deducted from the first ones, and still three and five liver parameters and seven and one kidney parameters at least appeared to be specifically linked to the GMO diet. We consecutively compared the parameters of GMO-fed rats to the six reference groups given other diets, focusing on the GMO effects as being more important than any other diet effects, and

**Table 3.** Effects of GMO treatments classified by organs

Parameters	Week	Sex	Dose	Control mean $\pm$ sem	GMO mean $\pm$ sem	Units
<b>Liver parameters</b>						
Albumin / Globulin Ratio	5	m	11%	1.782 $\pm$ 0.053	1.974 $\pm$ 0.043	Ratio
Albumin / Globulin Ratio	14	f	11%	2.334 $\pm$ 0.085	1.914 $\pm$ 0.083	Ratio
Albumin	5	f	33%	4.600 $\pm$ 0.054	4.850 $\pm$ 0.056	g/dl
Albumin	14	f	11%	5.130 $\pm$ 0.104	4.830 $\pm$ 0.091	g/dl
Globulin	5	m	11%	2.450 $\pm$ 0.090	2.150 $\pm$ 0.072	g/dl
Globulin	5	f	11%	2.110 $\pm$ 0.041	2.300 $\pm$ 0.080	g/dl
Globulin	14	f	11%	2.220 $\pm$ 0.080	2.560 $\pm$ 0.097	g/dl
Alanine aminotransferase	14	m	11%	67.100 $\pm$ 11.078	47.300 $\pm$ 1.422	u/l
Total protein	14	m	11%	7.140 $\pm$ 0.092	6.810 $\pm$ 0.099	g/dl
Total protein	14	m	33%	6.860 $\pm$ 0.090	7.1778 $\pm$ 0.112	g/dl
Triglycerides	5	f	33%	39.300 $\pm$ 1.578	54.900 $\pm$ 3.743	mg/dl
Triglycerides	14	f	11%	40.900 $\pm$ 3.889	50.900 $\pm$ 2.479	mg/dl
Liver weight	14	f	11%	7.250 $\pm$ 0.116	7.789 $\pm$ 0.163	g
Liver / brain ratio	14	f	11%	3.664 $\pm$ 0.059	3.890 $\pm$ 0.085	Ratio
<b>Kidney parameters</b>						
Creatinin	14	m	33%	0.520 $\pm$ 0.013	0.589 $\pm$ 0.031	mg/dl
Creatinin	14	f	11%	0.560 $\pm$ 0.016	0.630 $\pm$ 0.021	mg/dl
Urine sodium	14	m	33%	26.980 $\pm$ 3.487	20.122 $\pm$ 5.699	meq/l
Urine sodium excretion	14	m	33%	0.290 $\pm$ 0.028	0.189 $\pm$ 0.020	meq/time
Urine chloride excretion	5	f	11%	0.220 $\pm$ 0.025	0.330 $\pm$ 0.042	meq/time
Urine chloride excretion	5	f	33%	0.150 $\pm$ 0.022	0.250 $\pm$ 0.037	meq/time
Urine potassium	5	m	11%	112.210 $\pm$ 13.860	151.000 $\pm$ 10.039	meq/l
Urine phosphorus	5	m	33%	166.970 $\pm$ 24.719	108.310 $\pm$ 7.922	mg/dl
Urine phosphorus	14	m	33%	119.120 $\pm$ 13.479	81.822 $\pm$ 10.468	mg/dl
Urea nitrogen	14	f	11%	13.200 $\pm$ 0.742	15.500 $\pm$ 0.792	mg/dl
Kidney weight	14	m	33%	3.446 $\pm$ 0.070	3.201 $\pm$ 0.078	g
Kidney / brain ratio	14	m	33%	1.600 $\pm$ 0.030	1.483 $\pm$ 0.034	Ratio
Kidney % body weight	14	m	33%	0.705 $\pm$ 0.015	0.667 $\pm$ 0.009	%
<b>Pancreas</b>						
Glucose	14	f	11%	103.300 $\pm$ 2.495	112.600 $\pm$ 3.497	mg/dl
Glucose	14	f	33%	105.300 $\pm$ 2.432	115.800 $\pm$ 2.476	mg/dl
<b>Bone marrow</b>						
Neutrophils	5	m	33%	0.860 $\pm$ 0.058	1.050 $\pm$ 0.054	$\times 10^3/\mu\text{l}$
Eosinophils	14	m	33%	0.130 $\pm$ 0.015	0.200 $\pm$ 0.024	$\times 10^3/\mu\text{l}$
Reticulocytes	14	f	33%	0.085 $\pm$ 0.015	0.041 $\pm$ 0.008	$\times 10^6/\mu\text{l}$
Reticulocytes % RBC	14	f	33%	1.040 $\pm$ 0.201	0.470 $\pm$ 0.092	%

Based on Table 2, all the parameters significantly different between GMO-fed rats and corresponding controls are represented by their crude means  $\pm$  SEM in exactly corresponding units. The differences were always  $p < 0.05$  or  $< 0.01$  to controls according to one or two asterisks in Table 2. The controls are submitted to a substantially equivalent isogenic maize with the same diet, with all other conditions (genetic, temperature, light, space of caging, and so on) are identical. The time of exposure (weeks 5 and 14 corresponding, respectively, to 4 and 13 weeks of GMO diet), the sexes (males: m, females: f), and the dose (11 or 33% of GM Bt maize MON 863 in the equilibrated diet) are indicated.

always for males and females, respectively, four and zero kidney parameters and one and two liver parameters remained significantly different in all cases.

The significant liver changes in the 11% GMO-fed male rats that had the lowest growth rate was a total serum protein decrease (5%), possibly linked to a globulin decrease (12%). In females, the triglycerides were specifically enhanced in the animals that had liver and body weight increases above normal. In fact, triglycerides increased by 24–40% in females (either at week 14, dose 11% or at week 5, dose 33%, respectively).

At the kidney level, phenomena corresponding to urine phosphorus and sodium excretions diminished in males by 31–35% (week 14, dose 33%) for the most important results significantly linked to the treatment in comparison to seven diets tested, whereas other diets enhanced sodium excretion in some instances (data not shown).

Moreover, for males, none of these significantly changed parameters were similar to the variations due to the composition of the diet. The effect of the GMO diet was concomitant with a kidney weight decrease.

Other sporadic effects on serum glucose, urine chloride excretion, or reticulocytes, depending on the sex or the dose, are apparent.

## Discussion

The statistical analysis used in the conclusion of Hammond *et al.* (2006) was only carried out for this experiment by the Monsanto statistics center. The goal of this experiment is to study the possible toxicological effects of introducing the genetic construction producing an insecticide into the maize; thus, it should be guaranteed that the only variability sources in

the results are related to the presence, or not, of this transgene apart from purely random effects. In a sense, the presence of the 6 reference groups fed with other commercial varieties of corn, which are not substantially equivalent (with more or less salts or sugars), introduces the simultaneous study of other parameters. Moreover, the reference groups representing 60 rats per sex, measured for their biological parameters, have been compared to 10 rats fed with 33% GMO, by Monsanto. We think that this difference in size favors the uncertainties. We thus preferred to separate the analysis first between the GMO groups and the control ones, and then between GMO groups and the reference groups, in contrast to Monsanto analysis.

Moreover, a study with 20 animals per group already has a limited power of discrimination. Consequently, we could consider possible toxic effects if several parameters are disturbed for the same organ in a non-negligible manner. Unfortunately, besides controls and references, only 40 rats per sex in a total of 400 animals have been given GMOs in this study, and only half of those have been analyzed for biochemical parameters, *i.e.*, 10 per dose and per sex after 5 and 14 weeks, as indicated.

The body weight growth variations, usually hardly modified by a normal diet with very little quantities of toxin, represent an important factor to follow. This study was absent from the statistical report of Monsanto. The significant variations were not tested by Hammond *et al.* (2006), although the 11% GMO males form the lowest curve after week 2. However, we clearly proved very significant differences in weight growths for both males and females, with a lower effect with the 11% diet in comparison to 33% and controls. This increase was over controls in females with the 33% diet, and under controls for the 11% diet given to males. This may be not only an indication of the dysfunction of several organs as shown in Table 3, but also a sex-dependent effect related to endocrine disruption and/or hormonal metabolism differences. Surprisingly, sexual hormones were not measured in these regulatory tests. This could have explained some of these observations. In fact, the results of Table 2 concur with signs of possible hepatorenal toxicity with a greater kidney sensitivity in males and liver sensitivity in females. A differential sensitivity for toxicants among sexes is usual, the hepatic detoxification being hormone-dependent, for instance.

The differences were significant even if the reference diets had specific effects between them, such as 8–23% differences in liver alkaline phosphatase, alanine or aspartate aminotransferase activities, or small different sodium chloride exchanges and urine volume, probably due to different lipid or salt contents in the diets (data not shown).

The GMO-linked differences are illustrated at an hepatic level by a protein or triglyceride metabolism disruption. It is known that some hepatotoxics, such as the drug metabolite hydrazine, may cause liver necrosis and steatosis with hypertriglyceridemia in the blood (Sarich *et al.* 1996). These changes may have differential thresholds according to the sex or hormonal status, as with classical reactions to hepatocarcinogens (Castelli *et al.* 1986; Pitot *et al.* 1989). Moreover, nothing in the protocol allowed the conclusion that the 11% or 33% GMO proportions chosen in the diets were in the linear portion of a dose–response curve, after intoxication by the Bt

protein, for instance. Some Bt toxins may cause human hepatotoxicity by a nonapoptotic mechanism (Ito *et al.* 2004), or hepatic lipid peroxidation in rats (Shaban *et al.* 2003). However, it should be emphasized that a pleiotropic metabolic effect due to insertional mutagenesis and independent of the new insecticide produced in the GMO cannot be excluded.

To interpret the kidney data, although we did not have access to the kidney slices after the Appeal Court, Hammond *et al.* (2006) from Monsanto published that there were small increases of focal inflammation, and tubular regenerative changes in this group, in comparison to controls. They commented on a small decrease of serum chloride. After questions from the regulators in Europe, two board-certified pathology experts, proposed by Monsanto and who re-examined the slides, concluded that a classic chronic progressive nephropathy, for which male rats are sensitive (Hard and Khan 2004), had an incidence of 18/20 in the MON863 male group, higher than in controls (14/20), even if this was not considered as relevant by Hammond *et al.* (2006). If all the data are taken together, and overall in regard to the specifically disturbed urine chemistry parameters at weeks 5 and 14 (Table 2), which were not indicated by Hammond *et al.* (2006), it could be concluded that a GM-linked male renal toxicity is observed in this work.

To explain the sporadic results observed in the blood, we have little data. However, it is known in some instances that Bt toxins may also perforate blood cells (Rani and Balaraman 1996).

In conclusion, the two main organs of detoxification, liver and kidney, have been disturbed in this study. It appears that the statistical methods used by Monsanto were not detailed enough to see disruptions in biochemical parameters, in order to evidence possible signs of pathology within only 14 weeks. Moreover, the experimental design could have been performed more efficiently to study subchronic toxicity, in particular with more rats given GMOs in comparison to other groups. Considering that the human and animal populations could be exposed at comparable levels to this kind of food or feed that has been authorized in several countries, and that these are the best mammalian toxicity tests available, we strongly recommend a new assessment and longer exposure of mammals to these diets, with cautious clinical observations, before concluding that MON863 is safe to eat.

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