

Monsanto Response: de Vendômois et al. 2009

(A Comparison of the Effects of Three GM Corn Varieties on Mammalian Health)

Regarding: MON 863, MON 810 and NK603

Assessment of Quality and Response to Technical Issues

Synopsis:

- The laboratory findings primarily related to kidney and liver function reflect the large proportion of tests applicable to these organ systems. This is not a defect in the design of the study, but simply the reality of biochemical testing - there are good clinical tests of these systems which are reflected in blood chemistry. The function of other organ systems is assessed primarily via functional assessment, organ weight, and organ pathology rather than through blood or urine biochemical assays.
- The authors apply a variety of non-standard statistical approaches. Each unique statistical approach and each comparison performed increases the number of statistically significant findings which will occur by chance alone. Thus, the fact that de Vendômois et al. find more statistically significant findings than reported in the Monsanto analysis is entirely expected. The question, which de Vendômois et al. fail to address, is whether these non-routine statistical tests contribute anything of value to a safety assessment. Do they help to ascertain whether there are biologically and toxicologically significant events? In our opinion (consistent with prior reviews of other publications from Seralini and colleagues) they do not.
- The authors undertake a complex “principle component analysis” to demonstrate that kidney and liver function tests vary between male and female rodents. This phenomenon is well-recognized in rodents (and, for that matter, humans) as a matter of gender difference. (This does not indicate any toxic effect, and is not claimed to do so by the authors, but may be confusing to those not familiar with the method and background.)
- De Vendômois et al. appear to draw from this a conclusion that there is a gender difference in susceptibility to toxic effects. While such differences are possible, no difference in susceptibility can be demonstrated by gender differences in normal baseline values. Utilizing this alleged difference in gender susceptibility, the authors proceed to identify statistically significant, but biologically meaningless differences (see next bullet) and to evaluate the extent to which these changes occur in males versus females.
- De Vendômois et al. fail to consider whether a result is biologically meaningful, based on the magnitude of the difference observed, whether the observation falls outside of the normal range for the species, whether the observation falls outside the range observed in various reference materials, whether there is evidence of a dose-response, and whether there is consistency between sexes and consistency among tested GM materials. These failures are similar to those observed in previous publications by the same group of authors.
- While the number of tests that are statistically significant in males versus females would ON AVERAGE be equal in a random distribution, this ratio will fluctuate statistically. The authors have not, in fact, demonstrated any consistent susceptibility between genders, nor have they demonstrated that the deviations from equality in regards to numbers of positive tests fall outside of expectation. For example, if you flip a coin 10 times, on average you will get 50% heads and 50% tails but it is not unusual to get 7 heads and 3 tails on a particular 10 tosses. If you do this over and over and consistently get on average 7 heads and 3 tails then there may be something different about the coin that is causing this unexpected result. However, de Vendômois et al. have not shown any such consistent difference.

- While de Vendômois et al. criticize the lack of testing for cytochrome P450, such testing is not routinely a part of any toxicity testing protocol. These enzymes are responsible for (among other things) the metabolism of chemicals from the environment, and respond to a wide variety of external stimuli as a part of their normal function. There is no rational reason to test for levels of cytochromes in this type of testing, as they do not predict pathology. De Vendômois et al. could have identified thousands of different elements, enzymes and proteins that were not measured but this does not indicate a deficiency in the study design since there is no logical basis for testing them.
- While de Vendômois et al. criticize the occurrence of missing laboratory values, the vast majority of missing values are accounted for by missing urine specimens (which may or may not be obtainable at necropsy) or by a small number of animals found in a deceased condition (which are not analyzed due to post-mortem changes). Overall, despite the challenges in carrying out such analyses on large numbers of animals, almost 99% of values were reported.
- The statistical power analysis done by de Vendômois et al. is invalid, as it is based upon non-relevant degrees of difference and upon separate statistical tests rather than the ANOVA technique used by Monsanto (and generally preferred). The number of animals used is consistent with generally applicable designs for toxicology studies.
- Prior publications by Seralini and colleagues in both the pesticide and GM crops arenas have been found wanting in both scientific methodology and credibility by numerous regulatory agencies and independent scientific panels (as detailed below).
- In the press release associated with this publication, the authors denounce the various regulatory and scientific bodies which have criticized prior work, and claim, in advance, that these agencies and individuals suffer from incompetency and/or conflict of interest. In effect, the authors claim that their current publication cannot be legitimately criticized by anyone who disagrees with their overall opinions, past or present.
- It is important to note that several scientific groups and regulatory agencies have reviewed this study, and reject and/or refute the claims of the authors:
 - The [French High Counsel on Biotechnology](#) (HCB) has considered both the de Vendômois (2009) and Seralini (2007) papers and has found that these papers make no useful contribution to the safety assessment.
 - The [Food Standards Australia New Zealand](#) (FSANZ) have also dismissed this study, stating, “Séralini and colleagues have distorted the toxicological significance of their results by placing undue emphasis on the statistical treatment of data, and failing to take other relevant factors into account.”
 - Most recently, the [European Food Safety Authority](#) (EFSA) has weighed in on the matter, concluding that the study “*provides no new evidence of toxic effects.*”

To summarize, as with the prior publication of Seralini et al. (2007), de Vendômois et al. (2009) use non-traditional and inappropriate statistical methods to reach unsubstantiated conclusions in a reassessment of toxicology data from studies conducted with MON 863, MON 810 and NK603. Not surprisingly, they assert that they have found evidence for safety concerns with these crops but these claims are based on faulty analytical methods and reasoning and do not call into question the safety findings for these products.

Non- Monsanto Responses to de Vendômois et al 2009:

Independent of the Monsanto response below, individual scientists, scientific bodies, and regulatory agencies have reviewed the study, and reject the claims of the authors:

- The [French High Counsel on Biotechnology](#) (HCB) has considered both the de Vendômois (2009) and Seralini (2007) papers and has found that these papers make no useful contribution to the safety

assessment. The original document is in French. An English translation performed by Monsanto is as follows:

“Summary and conclusion

The approach followed by J. Spiroux de Vendômois et al. focuses on statistical differences between the various genetically modified maize and isogenic controls or commercial varieties.

In this publication, only a list of differences is listed without attempts to have biological or toxicological interpretation. As recurrently pointed out by international institutions responsible for the evaluation of toxicological risks, a significant statistical difference does not necessarily imply a conclusion of the existence of a biological disorder. Consequently, the argument of counting significant differences between test and control animals is not considered acceptable. Moreover the observed differences only apply to one sex, one observation time and one time of exposure. No hypothesis is presented to demonstrate that these sex-dependent variations are related to endocrinal variations. Additionally certain variations cited are contrary to what is generally accepted in cited literature concerning toxic effects notably on the liver or kidneys. All the approximations, insufficiencies or errors of interpretations made by Spiroux de Vendômois et al. do not allow concluding any hematologic, hepatotoxic or nephrotoxic effect of the three GMOs re-analysed from the initial data from the applicant [Monsanto]. To conclude, the HCB indicates that the Spiroux de Vendômois et al. (2009) as well as the preceding study (Séralini et al., 2007) do not bring any new scientific element to the evaluation of the three GMOs.

Nota bene:

It should be noted that the absence of conflict of interest of authors mentioned at the end of the article could be discussed. On January 5, 2010, the public website of the organization linked to the authors still present studies that pretend to demonstrate negative effects of MON810 like the Austrian study of 2008. These results were recognized as wrong by the authors themselves.”

- The [Food Standards Australia New Zealand](#) (FSANZ) have also dismissed this study, stating:
 - *In their latest paper, Séralini and colleagues again use a statistical analysis approach to interpret data from animal toxicity studies. On this occasion, they apply their methodology to separate feeding studies in rats with GM corn lines MON863, MON810 and NK603, and claim that their analysis has identified “new side effects linked with GM maize consumption, which were sex- and often dose-dependent”.*
 - *The authors claim that their results show “signs of toxicity” mostly associated with the kidney and liver, although other effects were reported to have been identified in heart, adrenal glands, spleen and haematopoietic system. Based on their reported findings, the authors argue strongly that longer-term (up to 2 years) feeding experiments are necessary in at least three animal species for in vivosafety evaluation of GM foods.*
 - *In response to Séralini’s 2007 paper, an expert scientific panel dismissed similar claims made by these authors. FSANZ also independently investigated the material presented in the paper and concluded that the incidence of statistically significant differences in animals fed GM corn (MON863) is entirely consistent with normal background variability.*
 - *In their most recent paper, Séralini and colleagues reject the consensus view and instead propose a cause-and-effect link between the findings and the new pesticides (herbicide or insecticide) specific to each GM corn, or associate the results with unintended effects arising from the genetic modification process itself. The authors do not offer any plausible scientific explanations for their hypothesis, nor do they consider the lack of concordance of the statistics with other investigative processes used in the studies such as pathology, histopathology and histochemistry.*
 - *Séralini and colleagues have distorted the toxicological significance of their results by placing undue emphasis on the statistical treatment of data, and failing to take other relevant factors into account. Reliance solely on statistics to determine treatment related*

effects in such studies is not indicative of a robust toxicological analysis. There is no corroborating evidence that would lead independently to the conclusion that there were effects of toxicological significance. FSANZ remains confident that the changes reported in these studies are neither sex- nor dose-related and are primarily due to chance alone.

- Most recently, the [European Food Safety Authority](#) (EFSA) has weighed in on the matter, concluding that the study “provides no new evidence of toxic effects:
 - *The EFSA GMO Panel has considered the paper by de Vendômois et al. (2009, A Comparison of the Effects of Three GM Corn Varieties on Mammalian Health, International Journal of Biological Sciences, 5: 706-726), a statistical reanalysis of data from three 90-day rat feeding studies already assessed by the GMO Panel (EFSA, 2003a,b; EFSA 2004a,b; EFSA 2009b,c). The GMO Panel concludes that the authors’ claims, regarding new side effects indicating kidney and liver toxicity, are not supported by the data provided in their paper. There is no new information that would lead it to reconsider its previous opinions on the three maize events MON810, MON863 and NK603, which concluded that there were no indications of adverse effects for human, animal health and the environment.*
 - *The GMO Panel notes that several of its fundamental statistical criticisms (EFSA, 2007a,b) of the authors’ earlier study (Seralini et al., 2007) of maize MON863 are also applicable to the new paper by de Vendômois et al. In the GMO Panel’s extensive evaluation of Seralini et al. (2007), reasons for the apparent excess of significant differences found for MON863 (8%) were given and it was shown that this raised no safety concerns. The percentage of variables tested reported by de Vendômois et al. that were significant for NK603 (9%) and MON810 (6%) were of similar magnitude to that for MON863.*
 - *The GMO Panel considers that de Vendômois et al.: (1) make erroneous statements concerning the use of reference varieties to provide estimates of variability that allow equivalence testing to place statistically significant results into biological context as advocated by EFSA (2008, 2009a); (2) do not use the available information concerning normal background variability between animals fed with different diets, to place observed differences into biological context; (3) do not present results using their False Discovery Rate methodology in a meaningful way; (4) give no evidence to relate well known gender differences in response to diet to claims of effects due to the respective GMOs; (5) estimate statistical power based on inappropriate analyses and magnitudes of difference. The significant differences highlighted by de Vendômois et al. have all been considered previously by the GMO Panel in its previous opinions on the three maize events MON810, MON863 and NK603.*
 - *The study by de Vendômois et al. provides no new evidence of toxic effects. The approach used by de Vendômois et al. does not allow a proper assessment of the differences claimed between the GMOs and their respective counterparts for their toxicological relevance because: (1) results are presented exclusively in the form of percentage differences for each variable, rather than in their actual measured units; (2) the calculated values of the toxicological parameters tested are not related to the normal range for the species concerned; (3) the calculated values of the toxicological parameters tested are not compared with ranges of variation found in test animals fed with diets containing different reference varieties; (4) the statistically significant differences did not show consistency patterns over endpoint variables and doses; (5) the inconsistencies between the purely statistical arguments of de Vendômois et al., and the results for these three animal feeding studies which relate to organ pathology, histopathology and histochemistry, are not addressed. Regarding claims made by de Vendômois et al. concerning the inadequacy of the experimental design of these three animal feeding studies, the GMO Panel notes that they were all carried out to agreed internationally-defined standards consistent with OECD protocols.*
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Monsanto Response to de Vendômois et al. 2009:

In the recent publication “A comparison of the effects of three GM corn varieties on mammalian health”, (de Vendômois et al., 2009), the authors claim to have found evidence of hepatorenal toxicity through reanalysis of the data from toxicology studies with three biotechnology-derived corn products (MON 863, MON 810 and NK603).

This theme of hepatorenal toxicity was raised in a previous publication on MON 863 by the same authors (Seralini et al., 2007). Scientists who reviewed the 2007 publication did not support that paper’s conclusions on MON 863 and the review addressed many deficiencies in the statistical reanalysis (Doull et al., 2007; EFSA, 2007a; EFSA, 2007b; Bfr, 2007; AFFSA, 2007, Monod, 2007, FSANZ, 2007). These reviews of the 2007 paper confirmed that the original analysis of the data by various regulatory agencies was correct and that MON 863 grain is safe for consumption based on the weight of evidence that includes a 90-day rat feeding study.

De Vendômois et al., (2009) elected to ignore the aforementioned expert scientific reviews by global authorities and regulatory agencies and again have used non -standard and inappropriate methods to reanalyze toxicology studies with MON 863, MON 810 and NK603. This is despite more than 10 years of safe cultivation and consumption of crops developed through modern biotechnology that have also completed extensive safety assessment and review by worldwide regulatory agencies, in each case reaching a conclusion that these products are safe.

General Comments:

De Vendômois et al. (2009) raise a number of general criticisms of the Monsanto studies that are worthy of mention before commenting on the analytical approach used by de Vendômois et al. and pointing out a number of examples where the application of their approach leads to misinterpretation of the data.

- 1) Testing for cytochrome P450 levels is not a part of any standard toxicology study, nor do changes in P450 levels per-se indicate organ pathology, as the normal function of these enzymes is to respond to the environment. Testing of cytochrome P450 levels is not part of any recognized standard for laboratory testing.
- 2) De Vendômois et al. note that the “effects” assessed by laboratory analysis were “mostly associated with the kidney and liver”. However, a review of the laboratory tests (annex 1 of paper), ignoring weight parameters, will indicate that measures of liver and kidney function are disproportionately represented among the laboratory tests. Urinary electrolytes are also particularly variable (see below). The apparent predominance of statistical differences in liver and kidney parameters is readily explained by the testing performed.
- 3) As noted by the authors, findings are largely within the normal range for parameters even if statistically significant, are inconsistent among GM crops, and are inconsistent between sexes. Despite this, and the lack of associated illness or organ pathology, the authors choose to interpret small random variations typically seen in studies of this type as evidence of potential toxicity.
- 4) The authors criticize the number of missing laboratory data, and indicate that the absence of values is not adequately explained. We would note that the bulk of missing values relate to urinalysis. The ability to analyze urine depends upon the availability of sufficient quantities of urine in the bladder at the time of necropsy, and thus urine specimens are often missing in any rodent study. Organ weights and other studies are generally not measured on animals found deceased (due to post-mortem changes the values are not considered valid). Each study consisted of 200 animals, or 800 possible data collections (counting urine, hematology, or organ weights + blood chemistry as one “type” as in the paper).
 - a. NK 603- of 600 possible data determinations, 28 values were missing. 20 were due to missing urines and 2 were missing weights and biochemical analysis due to animals found dead (1 GM, 1 reference). Of the remaining 6 values (hematology), only 1 value is from the GM-fed group.
 - b. MON 810- Of 600 possible determinations, 24 values were missing. 18 were due to missing urines and 1 value was missing (weight and biochemical analysis) due to an animal found dead (reference group). Of the remaining 5 values (hematology), 2 are from the GM-fed group and 3 from various reference groups.

- c. MON 863- Of 600 possible determinations, 25 values were missing. 13 were due to missing urines. 9 hematology analyses (3 GMO-fed) and 3 organ weight/biochemical analyses due to deaths (1 GMO) were reported as missing (not deceased).
 - d. These are large and complex studies. Ignoring urines and the small number of animals found deceased (which occurs in any large study), 20 data sets (17 hematology, 3 organ weights/chemistry) are missing from a possible 1800 sets, i.e.- almost 99% of data were present, despite the technical difficulties inherent in handling large numbers of animals.
- 5) The “findings” in this study are stated to be due to “either the recognized mutagenic effects of the GM transformation process or to the presence of... novel pesticides.” We would note that there is no evidence for “mutagenic effect” other than stable gene insertion in the tested products. We would also note that while the glyphosate tolerant crop (NK603) may indeed have glyphosate residues present, this is not a “novel” pesticide residue. The toxicity of glyphosate has been extensively evaluated, and the “effects” with NK603 cannot be explained on this basis. Similarly, other available data regarding the Bt insecticidal proteins in MON 810 and MON 863 do not support the occurrence of toxic effects due to these agents.

Statistical Analysis Approach:

De Vendômois et al., (2009) used a flawed basis for risk assessment, focusing only on statistical manipulation of data (sometimes using questionable methods) and ignoring consideration of other relevant biological information. By focusing only on statistical manipulations, the authors found more statistically significant differences for the data than was previously reported and claimed that this is new evidence for adverse effects. As is well documented in toxicology textbooks (e.g., Casarett and Doull, Toxicology, The Basic Science of Poisons, Klaassen Ed., The McGraw-Hill Companies, 2008, Chapter 2) and other resources mentioned below, interpretation of study findings involves more than statistical manipulations, one has to consider data in the context of the biology of the animal. This subject was addressed by a peer review panel of internationally recognized toxicologists and statisticians who reviewed the Seralini et al., (2007) publication. They state in Doull et al. (2007)

“The Panel concludes that the Seralini et al. (2007) reanalysis provided no evidence to indicate that MON 863 was associated with any adverse effects in the 90-day rat study (Covance, 2002; Hammond et al., 2006). In each case the statistical findings reported by both Monsanto (Covance, 2002; Hammond et al., 2006) or Seralini et al. (2007) were considered to be unrelated to treatment or of no biological or clinical importance because they failed to demonstrate a dose– response relationship, reproducibility over time, association with other relevant changes (e.g., histopathology), occurrence in both sexes, difference outside the normal range of variation, or biological plausibility with respect to cause-and-effect”

There are numerous ways to analyze biological data and a multitude of statistical tools. To provide consistency in the way that toxicology data are analyzed, regulatory agencies have provided guidance regarding the statistical methods to be used. The aforementioned peer review panel stated:

“The selection of the types of statistical methods to be performed is totally dependent upon the design of the toxicology study, and on the questions expected to be answered, as discussed in the US FDA Redbook (FDA, 2000). Hypothesis testing statistical analyses as described by WHO (1987), Gad (2001), and OECD (2002b) include those tests that have been traditionally conducted on data generated from rodent 90 -day and chronic toxicity studies. These are also the procedures that have been widely accepted by regulatory agencies that review the results of subchronic and/or chronic toxicity tests as part of the product approval process. There are many other statistical tests available such as 2k factorial analysis when k factors are evaluated, each at two levels, specific dose–response contrasts, and generalized linear modeling methods, but these methods typically have not been used to evaluate data from toxicology studies intended for regulatory submissions”

Commenting on the statistical analysis used originally to analyze the toxicology data for MON 863 conducted at Covance labs, the expert panel also stated:

“All of these statistical procedures are in accordance with the principles for the assessment of food additives set forth by the WHO (1987). Moreover, these tests represent those that are used commonly by contract research organisations throughout the world and have generally been

accepted by FDA, EFSA, Health Canada, Food Standards Australia New Zealand (FSANZ), and the Japanese Ministry of Health and Welfare. In fact, EFSA (2004) in their evaluation of the Covance (2002) study noted that it 'was statistically well designed'."

de Vendômois et al., (2009) selected non-traditional statistical tests to assess the data and failed to consider the entire data set in order to draw biologically meaningful conclusions. Their limited approach generated differences that, while being statistically significant, are insufficient to draw conclusions without considering the broader dataset to determine whether the findings are biologically meaningful. In Doull et al., (2007) the expert panel clearly stated:

"In the conduct of toxicity studies, the general question to be answered is whether or not administration of the test substance causes biologically important effects (i.e., those effects relevant to human health risk assessment). While statistics provide a tool by which to compare treated groups to controls; the assessment of the biological importance of any "statistically significant" effect requires a broader evaluation of the data, and, as described by Wilson et al. (2001), includes:

- *Dose-related trends*
- *Reproducibility*
- *Relationship to other findings*
- *Magnitude of the differences*
- *Occurrence in both sexes."*

Doull et al., (2007) raised questions regarding the appropriateness of some of the statistical analyses described in Seralini et al., (2007):

"The statistical analyses of the serum biochemistry, haematological, and clinical chemistry data conducted by Seralini et al. (2007) and by Monsanto were similar in concept as both used testing for homogeneity of variance and various pair-wise contrasts. The principle difference was that Seralini et al. (2007) did not use an ANOVA approach. The use of t- tests in the absence of multiple comparison methods may have had the effect of increasing the number of statistically significant results (emphasis added). The principle difference between the Monsanto and Seralini et al. (2007) analyses was in the evaluation of the body weight data. Monsanto used 'traditional' ANOVA and parametric analyses while Seralini et al. (2007) used the Gompertz model to estimate body weight as a function of time. The Gompertz model assumes equal variance between weeks, an assumption unlikely to hold with increasing body weights. While not inappropriate, as previously stated the Gompertz model does have limitation with respect to the interpretation of the results since it was not clear from the published paper whether Seralini et al. (2007) accounted for the changing variance and the correlated nature of the body weight data over time (emphasis added)."

Based on the expert panel conclusions in Doull et al., (2007); the statistical analysis used by, and the conclusions reached in, the de Vendômois et al. (2009) publication need to be carefully assessed. The authors use of inappropriate statistical methods in the examples below illustrate how inadequate analyses underpin the false and misleading claims found in de Vendômois et al., (2009).

Inappropriate use of False Discovery Rate method. De Vendômois et al., (2009) conducted t-test comparisons among the test and control and then applied the False Discovery Rate (FDR) method to adjust the p-values and hence the number of false positives. The FDR method is similar to many of the multiple comparison procedures that are available for controlling the family-wise error rate. Monsanto did not use any procedures for controlling the percentage of false positives for two reasons: (1) preplanned comparisons were defined that were pertinent to the experimental design and purpose of the analysis, i.e., it was not necessary to do all pairwise comparisons among the test, control, and reference substances and; (2) to maintain transparency and to further investigate all statistically significant differences using the additional considerations (Wilson et al, 2001) detailed above.

Inappropriate power assessment method. De Vendômois et al., (2009) claim that the Monsanto study had low power and support their claim with an inappropriate power assessment that is based on a simple t-test comparison of the test and control using an arbitrary numerical difference. This type of power assessment is incorrect because Monsanto used a one-way ANOVA, not a simple t-test. The appropriate power assessment should be relative to

the ANOVA and not a simple t-test. In addition, an appropriate power assessment should be done relative to the numerical difference that constitutes a biologically meaningful difference.

Other non-traditional statistical methods. De Vendômois et al., (2009) also claim that Monsanto did not apply the described statistical methods and simply used a one-way ANOVA and contrasts. This is a false statement since Monsanto used Levine's test to check for homogeneity of variances and if the variances were different the one-way ANOVA was conducted on the ranks rather than the original observations, *i.e.*, Kruskal-Wallis test.

Specific examples of flawed analysis and conclusions.

De Vendômois et al., (2009) have compared the results across toxicology feeding studies with three different biotech crops using some of the same statistical tests that were used in the previous publication (Seralini et al, 2007). Each of these biotech crops (MON 863, MON 810, NK603) are the result of unique molecular transformations and express different proteins. De Vendômois et al., (2009) claims that all three studies provide evidence of hepatorenal toxicity by their analysis of clinical pathology data only. One might anticipate, if these claims were true, that similar changes in clinical parameters could be observed across the three studies and that the changes observed would be diagnostic for kidney and liver toxicity and would be accompanied by cytopathological indications of kidney or liver disease. However, as shown in Tables 1 and 2 in Vendômois et al., (2009), the statistically significant "findings" in clinical parameters are different across studies, suggesting that these are more likely due to random variation (type one errors) rather than due to biologically meaningful effects. Moreover, as indicated below, there is no evidence of any liver and kidney toxicity in these studies, particularly in relation to other data included in the original study reports that is not mentioned in Vendômois et al., (2009).

NK603 - Kidney

For the NK603 study (Table 1), de Vendômois et al., (2009) listed data from some of the measured urinary electrolytes, urinary creatinine, blood urea nitrogen and creatinine, phosphorous and potassium as evidence of renal toxicity. It has been pointed out that urinalysis may be important if one is testing nephrotoxins (Hayes, 2008), particularly those that produce injury to the kidney. However, it has also been noted that "Urinalysis is frequently of limited value because the collection of satisfactory urine samples is fraught with technical difficulties" (Hayes, 2008). There was a lot of variability for some of the urinary electrolytes as indicated by the high standard deviations that may be attributed to the technical difficulties in collecting satisfactory urine samples.

Examining the original kidney data for NK603, the urine phosphorous values are generally comparable for 11% and 33% NK603 males and the 33% reference groups, while the 33% controls are generally lower than all groups. For females, 33% control females also had slightly lower phosphorous values, but they were not statistically different from 33% NK603 females, unlike males where the 33% NK603 male value was statistically different (higher) than 33% controls. When the blood phosphorous values were compared, there was a slight, but statistically significant reduction in 33% NK603 males compared to controls (but not references) at week 5, and there were no statistically significant differences in NK603 male and female blood phosphorous levels when compared to controls at the end of the 14 week study.

There were no statistically significant differences in urine sodium in males at weeks 5 and 14 in the original analysis (in contrast to the reanalysis reported by de Vendômois et al., 2009). As with phosphorous, there was considerable variability in urine sodium across all groups. The same results were observed for females. In addition, blood sodium levels for 11 and 33% NK 603 males and females were not different from controls. It is apparent when reviewing the data in the table below that the measured urinary electrolytes for the NK603 groups were similar to the values for reference, conventional (e.g., non-GM) corn groups.

Looking at the other parameters listed in Table 1 (de Vendômois et al., 2009), while there was a slight increase in urine creatinine clearance in 33% NK603 males at the interim bleed at week 5 compared to the controls and reference population, this was not apparent at the end of the study when the rats had been exposed longer to the test diets. There was no difference in urine creatinine levels in males. Blood creatinine levels were slightly, but statistically significantly lower in high dose males compared to controls at week 5. Increases in creatinine, not reductions are associated with renal toxicity. The same response was observed for serum urea nitrogen, a slight reduction at week 5 and no differences at in male blood creatinine or urea nitrogen at the end of the study. BUN, like creatinine, is not a very sensitive indicator of renal injury" (Hayes, 2008). Thus the small differences in BUN and serum and urine creatinine are not suggestive of kidney injury.

There was no evidence of changes in other urinary parameters such as pH, specific gravity, protein, sodium, calcium, chloride, volume and kidney weights. The most important factor relating to the kidney that de Vendômois

et al., (2009) did not consider was the normal microscopic appearance of the kidneys of rats fed NK603 grain. There was no evidence of treatment-related renal pathologic changes that the authors ignored in their risk assessment, a critical biological factor that an objective, scientific assessment would have considered.

MON 810 - Kidney

If Table 2 in de Vendômois et al., (2009) is examined, none of the aforementioned “findings” listed in Table 1 for NK603 are consistent except for blood urea nitrogen. Kidney weight data was listed, but this was not included in Table 1 for NK603. If the hypothesis of renal toxicity is correct, it is scientifically reasonable to have expected to observe at least some of the same “findings” between studies. The fact that there were no common findings supports the original conclusions reached by the investigative laboratory (and supported by regulatory agency review of these studies) that there is no evidence of kidney toxicity in rats fed either MON 810 or NK603 grain. Indeed, the data alleged by de Vendômois et al., (2009) to be indicative of kidney findings are more attributable to random variation that is commonly observed in rodent toxicology studies, which is well discussed in publications such as Doull, et al., (2007).

In Table 2, de Vendômois et al., (2009) highlights absolute kidney weights for males as being suggestive of kidney toxicity. The scientific basis for this assertion is unclear because there is no differences in male or female kidney weights (absolute, relative to body weight or brain weight) as shown in the table below:

De Vendômois et al., (2009) also lists blood urea nitrogen as indicative of kidney toxicity, yet there were no statistically significant differences in either MON 810 males or females when compared to controls (Hammond et al., 2006). In the absence of any other changes in urine or blood chemistry parameters that could be suggestive of kidney toxicity, and in consideration of the normal histologic appearance of kidneys of rats fed MON 810 grain, there is no scientific data to support the assertion of kidney toxicity in MON 810 fed rats.

NK603/MON 810 liver

Although de Vendômois et al., (2009) lists “findings” in Table 1 and 2 as being indicative of liver toxicity, analysis of these “findings” does not support this conclusion. There are no common “findings” in the liver between both studies. For NK603 de Vendômois et al., (2009) listed liver weights and serum alkaline phosphatase; for MON 810, serum albumin and albumin/globulin ratio. For NK603, the original analysis did not demonstrate statistical differences in absolute or, relative (to body or brain) liver weights for NK603 males and females compared to controls. Therefore, the statistical differences cited by de Vendômois et al., (2009) must be owing to the non-traditional statistical methods being used for their reanalysis of liver weight data. In regard to serum alkaline phosphatase, there were no differences for NK603 males or females when compared to controls; again de Vendômois et al., (2009) report statistical differences, but examination of the original data shows that the values for NK603 males and females are similar to controls and well within the range of values for the reference controls. There were no other associated changes in other liver enzymes, bilirubin, or protein that would be changes associated with liver toxicity. Lastly, but most importantly, the microscopic appearance of NK603 male and female livers was within normal limits for rats of that age and strain; therefore there was no evidence of liver toxicity. Similarly for rats fed MON 810, the only findings de Vendômois et al., (2009) list to support a conclusion of liver toxicity was albumin and albumin/globulin ratios. Contrary to the analysis in Table 2 of de Vendômois et al., (2009), there were no statistically significant differences in male or female serum albumin levels based on the original analysis. There were similarly no statistically significant differences in albumin/globulin with the exception of a slight decrease for 11% MON810 females when compared to controls at week 5. There were no differences observed at week 14 when the rats had been on test diets longer, nor were the differences dose related as they were not apparent in 33% MON 810 females relative to controls. The numerical values for serum albumin and albumin/globulin for MON 810 males and females were also similar to values for the reference groups. Consistent with NK603 rats, there were no other changes in serum liver enzymes, protein, bilirubin, etc., that might be associated with liver toxicity. The liver weights also appeared within normal limits for rats of the same strain and age used, again, consistent with a conclusion of no evidence of liver toxicity. In summary, no experimental evidence supports the conclusion for liver toxicity in rats fed NK603 and MON 810 grain as claimed by de Vendômois et al., (2009).

Kinetic plots

De Vendômois et al., (2009) has also presented some kinetic plots showing time-related variations for selected clinical parameters chosen for discussion. For 11% (low dose) control fed females, this publication reports that

there is a trend for decreasing triglyceride levels over time (week 5 compared to week 14) whereas for 11% MON 863 fed rats, levels increase slightly during the same time period. It is unclear why this publication used these complicated figures to assess these data sets since the same time course information can be obtained by simply comparing the mean data for the group at the two time points. Using this simpler method to assess the data, low dose control triglycerides dropped from a mean of 56.7 at week 5 to 40.9 at week 14. Low dose MON 863 female triglycerides increased slightly from 50.2 to 50.9. What de Vendômois et al., (2009) fails to mention is that high dose control female triglyceride levels increased from 39.3 at week 5 to 43.9 at week 14 and high dose MON 863 triglyceride levels decreased from 54.9 to 46.7. These trends are opposite from what occurred at the low dose, and the low dose trends are, therefore, not dose related. For the female reference groups, triglycerides went either up or down a bit between weeks 5 to 14, illustrating that these minor fluctuations occur naturally. Since most of the other figures reported were for the low dose groups, the trend for the high dose was sometimes opposite to that observed at the low dose. In summary, none of this analysis changes the conclusion of the study that there were no treatment-related adverse effects in rats fed MON 863 grain.

Summary

To summarize, as with the prior publication of Seralini et al, (2007), de Vendômois et al., (2009) uses non-traditional statistical methods to reassess toxicology data from studies conducted with MON 863, MON 810 and NK603 to reach an unsubstantiated conclusion that they have found evidence for safety concerns with these crops. As stated by the expert panel that reviewed the Seralini et al 2007 paper (Doull et al., 2007) “In the conduct of toxicity studies, the general question to be answered is whether or not administration of the test substance causes biologically important effects (i.e., those effects relevant to human health risk assessment). While statistics provide a tool by which to compare treated groups to controls; the assessment of the biological importance of any “statistically significant” effect requires a broader evaluation of the data, and, as described by Wilson et al. (2001), includes:

- Dose-related trends
- Reproducibility
- Relationship to other findings
- Magnitude of the differences
- Occurrence in both sexes.

A review of the original data for clinical parameters, organ weights and organ histology also found no evidence of any changes suggestive of hepato/renal toxicity as alleged in the de Vendômois et al., (2009) publication. This same publication also made false allegations regarding how Monsanto carried out their statistical analysis which has been addressed above.

Although there are many other points that could be made in regards to de Vendômois et al., (2009), given the fact that these authors continue to use the same flawed techniques despite input from other experts, it is not worthwhile to exhaustively document all of the problems with their safety assessment. Most importantly, regulatory agencies that have reviewed the safety data for MON 863, MON 810 and NK603 (including data from the 90 day rat toxicology studies reassessed by de Vendômois et al., 2009) have, in all instances, reached a conclusion that these three products are safe for human and animal consumption and safe for the environment. Peer reviewed publications on 90 day rat feeding studies with NK603, MON 810 and MON 863 grain have also concluded that there are no safety concerns identified for these three biotechnology-derived crops.

Additional Background:

Over the last five years, Seralini and associated investigators have published a series of papers first regarding glyphosate and later regarding Genetically Modified Organisms (GMOs, specifically MON 863). Reviews by government agencies and independent scientists have raised questions regarding the methodology and credibility of this work. The paper by de Vendômois et al. (December 2009) is the most recent publication by this group, and continues to raise the same questions regarding quality and credibility associated with the prior publications.

Seralini and his associates have suggested that glyphosate (the herbicide commonly referred to as “Roundup”™, widely used on GM crops (Roundup Ready™ and others) is responsible for a variety of human health effects. These allegations were not considered to be valid human health concerns according to several regulatory and technical reviews. Claims of mammalian endocrine disruption by glyphosate in Richards et al. (2005) were

evaluated by the Commission d'Etude de la Toxicité (French Toxicology Commission), which identified major methodological gaps and multiple instances of bias in arguments and data interpretation. The conclusion of the French Toxicology Commission was that this 2005 publication from Seralini's laboratory served no value for the human health risk assessment of glyphosate. A subsequent paper from Seralini's laboratory, Benachour et al. (2009), which was released via the internet in 2008, was reviewed by the Agence Française de Sécurité Sanitaire des Aliments (AFSSA, the French Agency for Food Safety). This review also pooled Richard et al (2005) and Benachour et al (2007) from Seralini's laboratory under the same umbrella of *in vitro* study designs on glyphosate and glyphosate based formulations. Again, the regulatory review detailed methodological flaws and questionable data interpretation by the Seralini group. The AFSSA final remarks of their review were "the French Agency for Food Safety judges that the cytotoxic effects of glyphosate, its metabolite AMPA, the tensioactive POAE and other glyphosate-based preparations put forward in this publication do not bring out any pertinent new facts of a nature to call into question the conclusions of the European assessment of glyphosate or those of the national assessment of the preparations". In August 2009, Health Canada's Pest Management Regulatory Authority (PMRA) published a response to a "Request for a Special Review of Glyphosate Herbicides Containing Polyethoxylated Tallowamine". The requester submitted 12 documents, which included the same claims made in the Benachour et al. (2009) publication. The PMRA response to this request concluded "PMRA has determined that the information submitted does not meet the requirements to invoke a special review," clearly indicating no human health concerns were raised in the review of those 12 documents in support of the request.

Regarding GMOs, Seralini et al. (2007) previously published a re-analysis of Monsanto's 90-day rat safety studies of MON863 corn. Scientists and regulatory agencies who reviewed the 2007 publication did not support that paper's conclusions on MON 863 and the review addressed many deficiencies in the statistical reanalysis (Doull et al., 2007; EFSA, 2007a; EFSA, 2007b; Bfr, 2007; AFFSA, 2007; Monod, 2007; FSANZ, 2007). These reviews of the 2007 paper confirmed that the original analysis of the data by various regulatory agencies was correct and that MON 863 grain is safe for consumption.

Using the MON 863 analysis as an example, Seralini et al. (2009) recently published a "review" article in the International Journal of Biological Sciences, claiming that improper interpretation of scientific data allowed sub-chronic and chronic health effects to be ignored in scientific studies of GMOs, pesticides, and other chemicals. This paper applies a complex analysis (principle component analysis) to demonstrate a difference in liver and kidney function between male and female rats. Despite the fact that these gender differences are well known and are demonstrated in control and GMO-fed animals, Seralini and his colleagues conclude that these normal findings demonstrate some type of sex-specific susceptibility to toxic effects. Based upon this reasoning, they proceed to over-interpret a variety of minor statistical findings in the MON 863 study. These very same conclusions were roundly criticized in 2007. In fact, the authors of this study admit that their observations "do not allow a clear statement of toxicological effects."

De Vendômois et al., (2009) elected to ignore the aforementioned expert scientific reviews by global authorities and regulatory agencies and again have used non-standard and inappropriate methods to reanalyze toxicology studies with MON 863, MON 810 and NK603. This is despite more than 10 years of safe cultivation and consumption of crops developed through modern biotechnology that have also completed extensive safety assessment and review by worldwide regulatory agencies, in each case reaching a conclusion that these products are safe.

Although some Seralini group publications acknowledge some funding sources, there are no acknowledgements of funding bias and conflict of interest. Financial support for Seralini's research includes the Committee for Research and Independent Information on Genetic Engineering (CRIIGEN) and the Human Earth Foundation. Seralini has been the Chairman of the Scientific Council for CRIIGEN since 1999. Seralini and this organization are known for their anti-biotechnology positions (<http://www.crii-gen.org/>). Both CRIIGEN and the Human Earth Foundation promote organic agriculture and alternatives to pesticides. It is interesting that over the last five years Seralini's group has published at least seven papers, four of which specifically target Monsanto's glyphosate-based formulations as detrimental to human health, and the remaining papers allege that Monsanto's biotechnology or GMO crops have human health implications. In addition, Seralini has a history of anti-Monsanto media releases and statements, including those on YouTube, reflecting not only Seralini's anti-Monsanto sentiment, but a lack of scientific objectivity. (<http://www.youtube.com/watch?v=HkRFgTyabSA> and http://www.youtube.com/watch?v=k_gF6gpSVdY)

Finally, it is worth noting the press release from CRIIGEN, issued at the time of release of the de Vendômois et al. publication:

"CRIIGEN denounces in particular the past opinions of EFSA, AFSSA and CGB, committees of European and French Food Safety Authorities, and others who spoke on the lack of risks on the tests which were conducted just for 90 days on rats to

assess the safety of these three GM varieties of maize. While criticizing their failure to examine the detailed statistics, CRIIGEN also emphasizes the conflict of interest and incompetence of these committees to counter expertise this publication as they have already voted positively on the same tests ignoring the side effects.”

This rather remarkable approach clearly indicates how far the authors of this publication have drifted from appropriate scientific discourse regarding GMO safety data. While they would reject criticisms of their methods and arguments by regulatory authorities and other eminent toxicology experts, most persons seeking an objective analysis will welcome broad expert input and a full assessment of the weight of evidence on the subject.

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