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Review

Report of an Expert Panel on the reanalysis by Séralini et al. (2007) of a 90-day study conducted by Monsanto in support of the safety of a genetically modified corn variety (MON 863)

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Abstract

MON 863, a genetically engineered corn variety that contains the gene for modified *Bacillus thuringiensis* Cry3Bb1 protein to protect against corn rootworm, was tested in a 90-day toxicity study as part of the process to gain regulatory approval. This study was reanalyzed by Séralini et al. who contended that the study showed possible hepatorenal effects of MON 863. An Expert Panel was convened to assess the original study results as analyzed by the Monsanto Company and the reanalysis conducted by Séralini et al. The Expert Panel concludes that the Séralini et al. reanalysis provided no evidence to indicate that MON 863 was associated with adverse effects in the 90-day rat study. In each case, statistical findings reported by both Monsanto and Séralini et al. 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. The Séralini et al. reanalysis does not advance any new scientific data to indicate that MON 863 caused adverse effects in the 90-day rat study.

Keywords: MON 863; Genetically modified organism; Rat; Subchronic toxicity; Statistical analysis; European Food Safety Authority (EFSA); Liver; Kidney

Abbreviations: ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BfR, *Bt, Bacillus thuringiensis*; BUN, blood urea nitrogen; CGB, French Commission du Génie Biomoléculaire; Covance, Covance Laboratories; DNA, deoxyribonucleic acid; EFSA, European Food Safety Authority; EU, European Union; FDA, US Food and Drug Administration; FSANZ, Food Standards Australia New Zealand; GM, genetically modified; GMO, genetically modified organisms; MOS, margins-of-safety; MON 863, YieldGard® Rootworm Corn; NPTII, neomycin phosphotransferase type II; OECD, Organization for Economic Cooperation and Development; PWG, Pathology Working Group; PMI, Purina Mills Inc.; RKI, Robert Koch Institute; SAS, Statistical Analysis System; US FDA, US Food and Drug Administration; US EPA, United States Environmental Protection Agency; WHO, World Health Organisation; US, United States.

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Contents

1. Introduction and background

MON 863 (YieldGard® Rootworm Corn[2]) was produced by insertion of deoxyribonucleic acid (DNA) sequences that encode a modified *Bacillus thuringiensis* Cry3Bb1 protein known to be selectively toxic to Coleopteran larvae, in particular, the corn rootworm. Corn rootworm is one of the most pernicious pests affecting maize crops around the world, and MON 863 offers farmers a way to effectively control this pest without the use of chemical insecticides. Related Cry proteins have been extensively tested and have shown no evidence of toxicity in animal studies (Sjoblad et al., 1992; McClintock et al., 1995; US EPA, 1998; WHO, 1999; Betz et al., 2000; Siegel, 2001; Federici, 2002), have a long history of safe use, have been granted a number of tolerance exemptions by the United States Environmental Protection Agency (US EPA) (including Cry3Bb1 protein – US EPA, 2004), and are approved in numerous other countries. The Cry3Bb1 protein showed no adverse effects in a high-dose (3200 mg/kg body weight) acute toxicity study in mice, consistent with other Cry proteins that have been tested in mammals (Sjoblad et al., 1992; Betz et al., 2000; Hammond et al., 2006). The genetic insert in MON 863 corn also contains coding for a selectable marker (*i.e.*, used to establish that the genetic construct is in fact present in the corn), neomycin phosphotransferase type II (NPTII). The European Food Safety Authority (EFSA) recently re-affirmed the safety of using NPTII as a selectable marker in biotechnology-derived crops (EFSA, 2007a).

In July of 2002, Monsanto Europe SA submitted an application to the European Union (EU) for MON 863 maize grain under Regulation (EC) No. 258/97 concerning Novel Foods and Novel Food Ingredients (EFSA, 2004). The MON 863 dossier, including the 90-day rat feeding study was assessed by the Robert Koch Institute (RKI), a government institute that evaluates regulatory dossiers in Berlin, Germany. Regarding the 90-day rat study, RKI stated: "From this extensive study, it can be deduced that even after long-term oral exposure to MON 863 maize kernels, no harmful effects are to be expected" (RKI, 2003).

The RKI assessment of the MON 863 dossier was circulated by the Commission to all EU Member States for review and comment. Numerous member states were supplied copies of the 90-day rat study report at their request. The French Commission du Génie Biomoléculaire (CGB) raised questions about a few findings in the rat study, including minor fluctuations in haematology parameters, *e.g.*, male white blood cell count, female blood glucose, male kidney weights, and the microscopic appearance of the male kidneys. Monsanto provided the CGB with a detailed analysis demonstrating that these fluctuations were within normal limits for rats of this age and strain. Monsanto's dossier and the RKI assessment were then transferred to EFSA for further review.

On April 18, 2004, the Scientific Panel on Genetically Modified Organisms of EFSA issued a scientific opinion on Monsanto's dossier for MON 863 (EFSA, 2004). The following conclusion was reached: "The Panel considers that the information available for MON 863 addresses the outstanding questions raised by the Member States and considers that MON 863 will not have an adverse effect on human and animal health or the environment in the

[2] YieldGard Rootworm Corn is a registered trademark of Monsanto Technology, LLC.

context of its proposed use.'' The Panel's report specifically discussed the findings from the 90-day rat study and they concluded: ''The results of these 90-day rodent studies do not indicate adverse effects from consumption of maize line MON 863...'' (EFSA, 2004).

Subsequent to issuance of EFSA's report, the French CGB concluded that based on further review of the Monsanto analysis, the aforementioned fluctuations in blood parameters were not considered to be biologically meaningful. However, the CGB requested more information to decide whether the male kidney findings were biologically relevant. The CGB suggested that Monsanto have an independent pathologic evaluation of the kidneys performed (*i.e.*, third party Expert Panel review). Monsanto organized a pathology peer review of the kidney histological slides and other data relevant to renal function by two expert pathologists, Drs. Gordon Hard and Andrea Terron. Neither pathologist is affiliated with Monsanto. Following their peer review of all clinical data relevant to kidney function including a blinded re-examination of the kidney tissue slides, these pathologists stated: ''It was concluded that dietary administration of MON 863 corn did not induce toxic effects in the kidneys of rats'' (Hard and Terron, 2004).

After reviewing the analysis of the relevant toxicological data by two independent pathology experts, and following review of additional data, the French CGB concluded that MON 863 maize had no toxic effect in the 90-day rat study (CGB, 2004). These additional assessments confirm the original study director and EFSA conclusions that MON 863 maize grain fed to rats produced no adverse health effects.

MON 863 maize has been approved for import and food use in many countries around the world, including the United States, Canada, Japan, Korea, Taiwan, the Philippines, Australia/New Zealand, China, the EU, Russia, Singapore, and Mexico.

Recently, a publication appeared in the journal Archives of Environmental Contamination and Toxicology, presenting a ''statistical re-evaluation'' of the individual animal data from the Monsanto 90-day rat study (Séralini et al., 2007). In this publication Séralini et al. (2007) infer that the statistical analyses conducted by Monsanto (Hammond et al., 2006) were inadequate as evidenced by the statement: ''The statistical methods used by Monsanto were not detailed enough to see disruption in biochemical parameters.'' Séralini et al. (2007) also state that their reanalysis demonstrated adverse effects of MON 863 when compared to the near-isogenic control (parental corn variety). Further Séralini et al. (2007) suggest that: (a) the Cry3Bb1 protein was not tested for human safety and that these class of proteins (*Bt* toxins) are toxic to human cells (*i.e.*, as evidenced by their statement: ''Some *Bt* toxins cause human hepatotoxicity by a non-apoptotic mechanism''), (b) the diets utilized in the reference groups (*i.e.*, six other varieties of non-genetically modified (GM) corn that were tested along with MON 863 and its near-isogenic control) did not have the same chemical composition and were not substantially equivalent to the near-isogenic control and GM diets; and, (c) Monsanto did not assess body weight variability.

This report has been prepared to address the inference in the Séralini et al. (2007) publication that the statistical analysis conducted by Monsanto (Hammond et al., 2006) was inadequate and that the reanalysis by Séralini et al. (2007) demonstrates evidence of hepatorenal toxicity. First, the internationally accepted procedures for the safety evaluation of biotech crops are reviewed and summarized (*i.e.*, the paradigm under which MON 863 was assessed by the European and other competent authorities). Second, a review and comment on the design, conduct and analysis of 90-day toxicity studies is presented. Third, the statistical procedures employed in the original 90-day study and those subsequently carried out by Séralini et al. (2007) are summarized and compared. Fourth, the interpretation of the resulting findings in the 90-day study, both as concluded in Monsanto's analyses (Hammond et al., 2006) and in the Séralini et al. (2007) study are discussed. This pertains in particular to the body weight, biochemical, haematological, and kidney weight data. Finally, conclusions with respect to the Monsanto and Séralini et al. (2007) analyses are presented.

2. Terms of reference, scope, and methodology

To address possible issues raised by the Séralini et al. (2007) publication, Cantox Health Sciences, Inc. convened an Expert Panel of toxicologists and statisticians from North America and Europe. The terms of reference for the Expert Panel included: (a) evaluate the statistical methods used by Séralini et al. (2007) and compare them to methods used in the original analysis (Hammond et al., 2006), (b) assess the biological importance of the statistical findings of the Hammond et al. (2006) and Séralini et al. (2007) publications, and (c) provide conclusions with respect to the results of the 90-day toxicity study. For their review, the Expert Panel was provided with a copy of the Séralini et al. (2007) and Hammond et al. (2006) publications as well as access to the raw data from the 90-day study. The Expert Panel also was provided with Monsanto's responses to questions from the French Commission du Génie Biomoléculaire (CGB) regarding the findings in the study (Hammond and Ward, 2004) as well as the report of the pathology working group which reviewed the relevant kidney findings in response to CGB questions (Hard and Terron, 2004). An initial draft report was prepared by Lynch and Munro of Cantox Health Sciences, Inc. This draft was carefully and critically reviewed by the Expert Panel and was subsequently modified and re-written following meetings of the Expert Panel members.

The Panel assessed only data relevant to the assessment of the statistically significant findings reported in the 90-day study by each of Monsanto (Hammond et al., 2006) and Séralini et al. (2007). The Panel did not evaluate the safety of MON 863 per se, since an evaluation of substantial equivalence, and of the genetic insert(s), was not done.

However, a section (Section 3) pertaining to the procedure by which these types of food products are assessed and approved is provided in this report to provide the reader with some perspective on the role of toxicity studies in the assessment of genetically modified foods. Similarly, a section (Section 4) on the general design and interpretation of 90-day toxicity studies, and of the types of statistics conventionally used in these studies, is provided as background material to facilitate an understanding of the Expert Panel's evaluation of the statistical findings reported in each of the Monsanto (Covance, 2002; Hammond et al., 2006) and Séralini et al. (2007) analysis of the data.

3. Internationally accepted methods for safety evaluation of genetically modified crops

The safety evaluation of GM crops intended for use as human food or animal feed involves the application of a well established set of criteria designed to critically examine all aspects of the safety of newly developed crops. Laboratory animal studies such as the one conducted by Hammond et al. (2006) on the GM variety MON 863 are intended to be used as a safety assurance tool and while such studies contribute to the overall safety evaluation, they are not the pivotal data upon which a conclusion of safety can be established.

In keeping with internationally recognized principles for the safety assessment of foods derived from GM crops (OECD, 1993, 2002a; FAO/WHO, 1996, 2000, 2003; MacKenzie, 2000; EC, 2003), the approach involves comparison of the newly developed food with a suitable comparator food which has a history of safe use. This concept, referred to as substantial equivalence or comparative analysis, includes a detailed comparison of agronomic features and composition of key nutrients, antinutrients, and natural toxicants of the new crop compared to the conventional counterpart. The purpose of this evaluation is to identify similarities and differences between the new variety and its comparators. Any differences then become the focus of the safety assessment.

The recommended approach for the safety of foods derived through biotechnology involves a thorough knowledge of the parent or traditional crop, molecular characterization of inserted DNA, evaluation of the safety of any proteins and other products expressed from the inserted DNA, application of the concept of substantial equivalence to identify similarities and differences in composition in comparison to suitable control conventional counterparts, and the evaluation of the safety and nutritional consequences of the intended alterations in composition and any other alterations identified (OECD, 1993, 2002a; FAO/WHO, 2000, 2003; Kuiper et al., 2001, 2002; Cockburn, 2003). It is a comprehensive evaluation of all the above pivotal data that leads to a successful safety assessment.

Considerable experience has been gained with the more than 50 GM crops that have been assessed by regulatory agencies, to state with confidence that the process of biotechnology as applied to date has not resulted in major unintended compositional changes in food or feed (ILSI, 2004). Indeed, as predicted, the application of biotechnology has resulted in minimal or no change in composition apart from the intended expression of specific traits.

Historically, toxicity tests in laboratory animals have played a significant role in ensuring the safety of chemicals present in foods, including food additives and contaminants that typically are consumed by humans in very small amounts. However, their value for assessing the safety of whole foods or major food constituents presents a number of significant challenges.

The difficulties encountered in assessing the safety of foods derived from GM crops in bioassays such as animal tests are well recognized (OECD, 1993, 2002a; LSRO, 1998; FAO/WHO, 2000, 2003). It has been pointed out on numerous occasions that animal feeding studies with whole foods or feeds must be designed and conducted with great care to avoid problems encountered with nutritional imbalance from overfeeding a single whole food, which itself can lead to adverse effects. In undertaking such tests, a balance must be struck between feeding enough of the test material to have the possibility of detecting a true adverse effect and, on the other hand, not inducing nutritional imbalance. Furthermore, the multiples over anticipated human intake one would like to attain in animal tests are simply not achievable for practical reasons, and, as result, lower margins of safety have to be accepted (WHO, 1987; Hattan, 1996; Munro et al., 1996).

That is why animal toxicity tests, while providing an additional measure of safety assurance, cannot be considered as pivotal data in making an overall safety assessment and a more focused and multidisciplinary approach as outlined above is required (FAO/WHO, 2003). Thus the reports of both Hammond et al. (2006) and Séralini et al. (2007) must be considered in the light of other data pertaining to safety of MON 863 and to do otherwise runs against the guidance and principles of national regulatory agencies and international organizations.

4. Design, conduct, and interpretation of toxicology studies conducted to support safety evaluation

As discussed in the previous section, the procedures by which biotechnology-derived foods are assessed do not require that toxicity studies be conducted; however, it is possible to test such foods/food ingredients, as well as traditional whole foods, in standard animal toxicity bioassays. The conduct of these studies is essentially similar to that employed for the subchronic testing of a conventional chemical. The main difference as previously noted, is that high margins-of-safety (MOS) between human exposure levels and possible effect levels in the rodent study cannot be achieved since whole foods, including biotechnology-derived foods, are macronutrients.

Animal studies on biotechnology-derived foods should be conducted according to the general guidance provided by the EFSA (2006). The EFSA documents recommend protocol and study designs which generally follow those established by the Organization for Economic Cooperation and Development (OECD) (1998) (Test Guideline 408, 90-Day Oral Toxicity Study in Rodents). Similar guidelines are available from the US Food and Drug Administration (FDA, 2000) and the World Health Organisation (WHO, 1987). The study design of the 90-day rat study on MON 863 was based on OECD Test Guideline 408 standards and was consistent with criteria established by WHO (1987) and the FDA (2000).

In regard to statistical analysis of the data generated from a 90-day subchronic toxicity study, OECD Test Guideline 408 states: "When applicable, numerical data should be evaluated by an appropriate and generally acceptable statistical method. The statistical methods and the data to be analyzed should be selected during the design of the study." While no specific statistical tests are prescribed, it is worthy to note the importance of the selection of the tests to be conducted at the design stage of the study, *prior to the initiation* of the study (FDA, 2000). This does not imply that statistical analyses cannot be done in a *post hoc* fashion. *Post hoc* analyses can provide insight into uncertain or questionable statistical or biological findings; however, tests conducted after study completion should appropriately reflect the original design and objectives of the study.

While, specific statistical tests to be employed in the analysis of the data from a 90-day study are not clearly spelled out in international guidelines, a number of publications are available in the scientific literature that address this topic (Weil, 1962, 1973, 1982; Williams, 1971, 1972; Weil and Gad, 1980; SOT, 1989; Gad, 1998; OECD, 2002b). In addition, WHO (1987) has provided some guidance with respect to this issue. WHO (1987) state that for continuous types of data (*e.g.*, body weight, clinical chemistry and haematology values) normality should be assessed along with homogeneity of variance (by Bartlett's test). If variance is heterogeneous, WHO (1987) recommends transforming the data (*e.g.*, applying a logarithm) or using non-parametric tests. Where variance is found to be homogenous (the most common case), WHO (1987) recommends the application of an analysis of variance testing in conjunction with individual pair-wise comparison tests (*e.g.*, Student's unpaired *t*-tests). For quantal data such as the incidence of pathological lesions, WHO (1987) has recommended application of a $2 \times k$ chi square test for assessment of incidence heterogeneity and Fisher's exact test for individual group comparisons. More recently, Gad (2001) has provided a detailed review of statistical tests commonly employed in hypothesis testing.

The selection of the types of statistical methods to be performed is totally dependent upon the design of the toxicology study, and of 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 generally 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 2^k 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.

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.

As an illustration, Wilson et al. (2001) state that a single statistically significant elevation in serum alanine aminotransferase (ALT) at one time point in a study is unlikely to be treatment-related, however, should serum aspartate aminotransferase (AST) also be elevated in conjunction with findings of hepatocellular necrosis, then one can reasonably conclude that the statistical finding of increased ALT is in fact treatment-related and biologically important.

Moreover, a statistically significant finding may not automatically constitute definitive evidence of an adverse or toxicologically important effect (Chan et al., 1982). The magnitude of departure from the normal range, the consistency of the out-of-range responses, and the relationships of the abnormal responses to the physiological, physical, biochemical, and metabolic well-being of an animal all have to be considered. It is very common in toxicology studies with limited group sizes to observe statistically significant differences in toxicological endpoints between treated and control groups. These must be evaluated on a case-by-case basis, taking into consideration such factors as the direction of change (*i.e.*, increase or decrease), evidence of a dose response, the presence of a similar effect in animals of the other sex, and the presence of other indicators of clinicopathology including, specific organ toxicity.

Also, given the number of comparisons between control groups and experimental groups undertaken in a toxicity

study, one can actually expect statistical differences to occur by chance alone. The WHO (1987) specifically notes the impact of multiple comparisons on the likelihood of obtaining a statistically significant response in any one dependent variable. According to WHO (1987), there exists a significant probability that one or more variables measured in control *versus* treated group comparisons will show statistical significance. For example, when using the $p \leqslant 0.05$ significance level, if 100 independent variables are measured in the control/treated group comparisons, then there is a 99.4% chance that at least one variable will show statistical significance by chance alone. With a p value of 0.05, upwards of 5 statistically significant comparisons between treated and control groups could be expected to occur randomly. This is referred to as Type I error or "false-positive" (Gad, 2001). Invariably, the biological importance of these "false positives" cannot be ascertained *a priori*. Only in the context of the entire dataset, and of historical control data, and through evaluation of dose–response, biological plausibility, causality, *etc*., can their true importance to safety assessment be evaluated.

5. Review and comment on methods used in the MON 863 90-day rat study and the reanalysis by Séralini et al. (2007)

5.1. Design of Monsanto 90-day toxicity study on MON 863

Hammond et al. (2006) presents the published version of the 90-day oral toxicity study conducted on MON 863 corn by Covance Laboratories (Covance, 2002). A complete report of the Covance study, including summary and individual animal data is available online through Monsanto's Website (http://www.monsanto.com/monsanto/content/products/technicalandsafety/fullratstudy.pdf).

The design of the rat study is based on the OECD Guideline No. 408 for a Repeated Dose 90-day Oral Toxicity in Rodents (OECD, 1998). This study was conducted in the United States at Covance Laboratories. It consisted of 10 groups of 20 Crl:(SD)CD IGS BR rats/sex/group obtained from Charles River Laboratories. A one-week pre-study baseline food consumption determination was conducted with Purina Mills Inc. (PMI) Rodent Chow #5002. PMI Rodent #5002 is used as the diet for many of the rodent toxicity studies conducted in the United States and other countries. PMI Rodent Chow #5002 comprises approximately 33% commercial maize grain (source of grain not specified). Therefore, the test and control diets for this study were formulated to compositionally match this standard rodent diet. MON 863 grain was formulated into the diets at 11% or 33%, with the lower dose level being supplemented with 22% commercial non-transgenic maize (*i.e*., conventional maize provided by PMI). Two control groups of rats were fed diets formulated with 11% or 33% non-transgenic corn (*i.e*., the "near-isogenic control") that has the same background genetics as MON 863 corn (except for the transgenes of interest). The 11% near-isogenic control diet was supplemented with the same 22% commercial non-transgenic maize added to the 11% MON 863 diet. Additional reference groups were fed diets containing 33% corn grain from six different commercially available non-transgenic varieties of maize. The purpose of including these additional reference groups was to establish the normal range of values for all the variables measured.

The test material for this study was produced by crossing inbred maize A634 containing the gene encoding for the Cry3Bb1 protein with conventional inbred maize LH82 to yield the MON 863 hybrid. The near-isogenic control hybrid was produced by crossing conventional maize A634 (not containing the gene encoding for Cry3Bb1) to conventional maize LH82. The formulated test and control diets were analyzed for normal maize constituents, pesticide residues, and mycotoxins. In addition, the control diets were ascertained to be free of GMO corn varieties. The results of the compositional analyses of test, control and reference diets are displayed in Table 1. It can be concluded from these analyses that the test diets were compositionally equivalent to the near-isogenic control and reference diets except for their trace quantities of transgenic DNA and the encoded proteins Cry3Bb1 and NPTII. All 10 formulated diets utilized on this study met the specifications established by PMI for Rodent Chow #5002. These data corroborate the results of two studies submitted to RKI which clearly establish the compositional equivalence of MON 863 grain and forage to conventional maize (Ridley et al., 2002; George et al., 2004). A 42-day broiler feeding study has established the nutritional equivalence of MON 863 grain to conventional maize grain (Taylor et al., 2003).

The design of this subchronic study included the collection of blood samples from 10 rats/sex/group at week 5 and at study termination. An autopsy, that included measurement of selected organ weights and histopathological

Table 1
Results of the compositional analyses of the MON 863, near-isogenic control, and reference diets

Diet	Protein (%)	kcal/100 g	Total fat (%)	Ash (%)	Crude fibre (%)
MON 863 (11%)	20.4	359	4.89	6.06	4.52
MON 863 (33%)	21.3	362	5.39	6.52	4.64
LH82 × A634 (11%)	20.8	358	4.97	6.67	4.49
LH82 × A634 (33%)	21.5	365	5.55	6.24	4.42
MON 847 (A1 × H99)	21.7	363	5.17	6.42	4.57
Asgrow RX-770	20.5	361	5.21	6.86	4.64
LH235 × LH185	21.0	363	5.16	6.11	4.52
LH200 × LH172	20.2	362	5.02	6.21	4.60
B73Ht × LH82	21.4	363	5.33	6.09	4.30
Burrus BX86	21.5	359	5.24	6.69	4.49
PMI #5002 diet specs	>20	310[a]	>4.5	<7.0	<5.5

[a] Average.

examinations, was performed on all 33% MON 863 and 33% near-isogenic control males and females at study termination. Body weight data, food consumption data, haematology results, serum chemistry, urinalysis and urine chemistry results, organ weight data, and incidence of microscopic findings were subjected to statistical analyses.

5.2. Statistical methods used by Monsanto

The 90-day MON 863 rat study was a randomized design with two treatment factors: groups (a test and a control at both 11% and 33% dietary concentrations and 6 reference groups) and sex (male and female) (Hammond et al., 2006).

The principal statistical analysis, which was done by sex, was a one-way analysis of variance. For responses satisfying the analysis of variance assumptions (Levene's tests, similar to Bartlett's test, but more reliable for data where moderate departures from normality can be expected), a parametric analysis of variance was done and comparisons of the test to the control at both 11% and 33% dietary concentrations were done using contrasts. An additional contrast was done to compare the mean of the 33% treatment to the mean of the references. A Fisher's protected LSD *t*-test was used for these contrasts. For responses not satisfying the analysis of variance assumptions, a Kruskal–Wallis non-parametric test was performed. Analysis of the pathology and histopathology data was conducted through the use of the Fisher's Exact Test. The statistical package used in the data analysis was SAS, release 8.2 (SAS, 1999). 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".

5.3. Statistical methods used by Séralini et al. (2007)

The statistical methods utilized by Séralini et al. (2007) are part of a *post hoc* re-evaluation of the raw data generated from the 90-day Covance (2002) study. Given the limited detail presented in the methods section of the paper it is difficult to clearly ascertain the exact nature of the statistical analyses conducted by Séralini et al. (2007).

Initially, Séralini et al. (2007) reanalyzed the raw data using the same procedures as employed in the Monsanto analysis (Hammond et al., 2006). This was apparently done to confirm the results reported in the Hammond et al. (2006) publication.

Séralini et al. (2007) carried out a statistical analysis of the serum biochemistry, haematology, and urinalysis data that utilized various parametric and non-parametric tests following assessment of the homogeneity of variance. Séralini et al. (2007) compared the 11% and 33% MON 863 groups with their respective controls and the 33% MON 863 group with the 6 reference groups, as did Hammond et al. (2006). In addition, Séralini et al. (2007) compared the 11% MON 863 group with the 6 reference groups. Specifically, while somewhat unclear, Séralini et al. (2007) appeared firstly to evaluate the normality of the datasets for each sex and dose level through the use of the Shapiro test and variance equality (homogeneity) by the *F*-test. The results of the analysis of normality and of variance equality were used to select the appropriate two-tailed comparison test (Crawley, 2005): an unpaired *t*-test, a Welch corrected *t*-test, or a Mann–Whitney test. Based on this information, no ANOVA tests appear to have been carried out. Since Séralini et al. (2007) did not make any adjustment for the multiple comparisons (11% MON 863 *versus* 11% control, 33% MON 863 *versus* 33% control, 11% MON 863 *versus* reference varieties, and 33% MON 863 *versus* reference varieties) within an endpoint, they report a greater number of statistically significant results although the descriptive statistics they obtained were identical to those reported in the original Covance (2002) study.

The statistical analysis of the body weight data by Séralini et al. (2007) differed appreciably from that employed in the Monsanto analyses (*i.e.*, establish variance homogeneity, one-way ANOVA, and parametric analyses) (Covance, 2002; Hammond et al., 2006). Séralini et al. (2007), according to their paper, appear to have conducted a multivariate analysis of the growth curve data by first fitting separate Gompertz models and then using the full and reduced model approach to compare the estimated parameters of the different models. The Gompertz model fitted was a non-linear model using weight as the dependent variable and weeks as the independent variable. The Gompertz model assumes equal variances for each week; however, for body weight data, this assumption does not hold, thus the results must be interpreted carefully since both "false-positives" or "false-negatives" could occur do to this limitation. In addition, some would criticize the characterization of the Gompertz modeling of the body weight data as a "multivariate analysis" rather than as a repeated measures analysis because an individual rat is being measured at each time point, the measurements across time are, and must be, correlated. It was not clear whether Séralini et al. (2007) accounted for the changing variance over time or for the correlated nature of the body weights of each individual rat.

6. A comparison and interpretation of the findings from the Monsanto and Séralini analyses

Table 2 presents the statistically significant differences between the MON 863 and the near-isogenic control treated rats that were observed after 14 weeks of treatment

Table 2
Week 14 data – comparison of statistical findings in the Monsanto and Séralini analyses

Parameter (units)	Sex	Dose (%)	Near-isogenic control	MON 863	Reference controls mean (range)[a]	Monsanto analysis[b]	Séralini analysis
WBC (10^3/μl)	M	33	8.6	10.4	8 (4.3–13)	**	
Lymphocyte (10^3/μl)	M	33	7.2	8.8	6.4 (3.5, 11.4)	**	
Basophils (10^3/μl)	M	33	0	0.3	0.01 (0.0–0.1)	**	
Eosinophils (10^3/μl)	M	33	0.13	0.2	0.2 (0.1–0.3)		*
Reticulocyte	F	33	0.09	0.04	0.07 (0.02–0.2)		*
Retic RBC	F	33	1	0.5	0.8 (0.2–2.5)		*
A/G ratio	F	11	2.3	1.9	2 (1.3–2.7)	**	**
Albumin (g/dl)	F	11	4.8	5.1	5 (4.1–5.7)		*
Globulin (g/dl)	F	11	2.2	2.6	2.6 (1.9–3.6)	**	*
ALT (U/l)	M	11	67	47	52 (31–127)		*
Total Prot. (g/dl)	M	11	7.1	6.8	7 (6.4–7.9)		*
Total Prot. (g/dl)	M	33	6.9	7.2	7 (6.4–7.9)		*
Triglycerides (mg/dl)	F	11	41	51	40 (27–63)	**	*
Glucose (mg/dl)	F	11	103	113	115 (93–143)	*	*
Glucose (mg/dl)	F	33	105	116	115 (93–143)	*	**
Creatinine (mg/dl)	M	33	0.52	0.59	0.55 (0.4–0.7)		*
Creatinine (mg/dl)	F	11	0.56	0.63	0.6 (0.5–0.8)		*
BUN (mg/dl)	F	11	13	16	15 (12–19)		*
Urine sodium (meq/l)	M	33	27	20	37 (5.1–89.2)		*
Urine sodium excretion (meq/time)	M	33	0.29	0.19	0.35 (0.1–0.7)		*
Urine phosp (mg/dl)	M	33	119	81	123 (10.7–298.5)		*
Organ weights							
Liver weight (g)	F	11	7.3	7.8	7.8 (5.1–10.4)		*
Liver/brain weight	F	11	3.7	3.9	3.9 (2.6–5.2)		*
Kidney weight (g)	M	33	3.4	3.2	3.3 (2.3–4.3)		*
Kidney/body	M	33	0.71	0.67	0.68 (0.55–0.86)		*
Kidney/brain	M	33	1.6	1.5	1.6 (1.2–2.0)		*

* Statistically significantly different at $p < 0.05$.
** Statistically significantly different at $p < 0.01$.
[a] Mean and minimum–maximum values for reference control groups.
[b] Statistical analyses involved initial ANOVA testing, followed by specific contrasts. Contrasts were evaluated for statistical significance only when ANOVA was statistically significant.

as reported by Monsanto (Covance, 2002; Hammond et al., 2006) and Séralini et al. (2007).

When comparing the data collected at week 14, where there were statistically significant differences in clinical parameters (haematology, chemistry, urinalysis), five were reported to achieve statistical significance in both the Monsanto (Covance, 2002; Hammond et al., 2006) and Séralini et al. (2007) analyses. For three other parameters, differences were apparent in the Monsanto analysis, but not in the Séralini et al. (2007) analysis. For 13 other parameters, the Séralini et al. (2007) analysis reported statistically significant differences, while the Monsanto analysis did not. Among the significant differences from controls for 33% MON 863 reported by Hammond et al. (2006), none was significantly different from the mean of the 6 reference varieties (except for white blood cells, lymphocytes and basophils) (Hammond et al., 2006).

Séralini et al. (2007) reported that there were five significant differences in organ weights. Since for the liver weight and liver/brain weight ratio significant differences occur only at the low concentration (11%) females, these results are not dose-related. The significant differences for kidney weight, kidney/body weight ratio, and kidney/brain weight ratio in the 33% males were not significantly different from the reference means. No statistically significant effects on the weights of any organ, either absolute or relative, were recorded by Monsanto (Covance, 2002; Hammond et al., 2006).

A number of statistically significant differences in biochemical, haematological, and urinalysis parameters noted by Séralini et al. (2007), but not found in any of the Monsanto analyses, occurred only at the low-dose level (*e.g.*, serum ALT, triglycerides, blood urea nitrogen (BUN), albumin, and urinary potassium excretion) in the 11% MON 863 treatment groups and not at the high-dose level (33% MON 863). As a result, these particular differences show no dose–response relationships, and, therefore, are not likely to be treatment-related.

In all cases (except basophil count) statistically significant differences listed in Table 2 were within the range of values reported in the reference groups. The reference groups are superior to the use of traditional historical controls since they are contemporaneous with the treated and near-isogenic control groups and were placed on study by the same laboratory, at the same time, and under the same conditions, as the original test (MON 863) and

near-isogenic control groups. These reference groups are useful in the interpretation of the findings in the MON 863 treated groups with respect to the near-isogenic controls. The use of additional contemporaneous reference or control groups to mitigate the effects of background noise has been commented on by the OECD (2002b). Specifically, OECD (2002b) state:

> "Because of normal biological variation in inter-animal values, and the alterations in values in response to a variety of inputs, evaluators have to contend with much "noise" in this area; they are frequently presented with statistically significant but scattered effects, in the absence of any evidence of clinically significant relationships with specific toxicity endpoints…. To deal with the noise it is necessary to examine whether an effect is within the normal range of variation, using concurrent and historical controls… Frequently these data show apparently random changes in individual groups, or, less commonly, trends in changes across groups that are unrelated to dose. If, as an aid to evaluation, historical control data are used for comparison, it must be kept in mind that "normal" values in haematological and clinical chemistry measurements depend on the specific methods used to generate the data. Thus, only values obtained using identical methods at the same laboratory are valid in such comparisons."

Séralini et al. (2007) discount the use of the reference groups by stating that: "…they did not have the same final chemical composition even if these diets also meet PMI specifications for Certified 5002 Rodent Diet" and "they were not demonstrated to be substantially equivalent to the GMO and control diet." However, Séralini's argument to dismiss the reference group diets is not correct. As Séralini et al. (2007) acknowledge, all diets met the PMI specifications for certified diets which means they have similar nutrient composition as shown in Table 1. All diets were formulated with the same batches of ingredients (fat, vitamin pre-mix, fibre, *etc.*). The only difference between them was the source corn grain that was used which was either MON 863, its near-isogenic control, or 1 of 6 different commercial, non-genetically modified corn varieties. All diets were formulated by PMI during the same week and tested concurrently in the toxicology testing laboratory (Covance, 2002).

6.1. Body weight

In the original analysis conducted by Monsanto (Covance, 2002; Hammond et al., 2006) there were no statistically significant differences in terminal body weight or body weight gains between the MON 863 and the near-isogenic control groups for either males or females. Numerically, the final body weight for males treated with 33% MON 863 was 502 g *versus* 515 g for the near-isogenic controls (−3% difference), while for females, the 33% MON 863 group final body weight was 289 g *versus* 278 g for the near-isogenic controls (+4% difference). The final body weight in males treated at the low dose (11% MON 863) was 496 g *versus* 513 g for the controls (−3% difference). In females, the respective low-dose group body weights were 286 g in the MON 863 group *versus* 278 g in the near-isogenic control (+3% difference). These minor fluctuations in body weights were not dose-related in males, and were opposite in direction in females, and, therefore, were not considered to be biologically important. Further, the mean of the 33% MON 863 group is not different from the mean of the 6 reference groups. The numerical changes in body weight were attributed to random variation. The Panel notes that visual inspection of the growth curves in the Hammond et al. (2006) publication confirms that MON 863 treatment did not affect body weight or body weight gain over the course of the study.

6.2. Clinical parameters

6.2.1. Haematology, clinical chemistry, and urinalysis

A detailed discussion of the lack of biological relevance of statistically significant differences in haematology, clinical chemistry, and urinalysis parameters was addressed by EFSA (2004) and Hammond et al. (2006).

EFSA (2004) concluded that statistically significant changes in several haematological and clinical chemistry parameters in the 90-day study were not biologically meaningful given the small magnitude of difference and that the ranges fell within those of the reference and/or control groups.

Hammond et al. (2006) noted that while a few statistically significant changes occurred in the clinical chemistry parameters, these differences were not considered of biological importance since they were either different from the 6 references, but not the controls, or were not dose- (33% *versus* 11%) or time-related (week 5 *versus* week 14 bleed). Similarly, the haematological changes were concluded to be of no biological importance since the changes were small, and the changes were not replicated in females and were not correlated with any histopathological changes in the spleen or lymph nodes (Hammond et al., 2006).

6.3. Kidney weight data

Upon post-mortem examination of the study animals, the kidney weights in high-dose males were found to be lower (*i.e.*, 7.1%) than the respective kidney weights in the control group (33% near-isogenic parental strain). This weight difference between high-dose and control animals was also evident when the male kidney weights were normalized for either body weight or brain weight. In the Monsanto analysis (Hammond et al., 2006), the kidney weights of the treated, isogenic controls and the 6 reference controls were analyzed by ANOVA testing. Since the results of the ANOVA were not statistically significant ($p = 0.521$), the kidney weight data were not analyzed further. Séralini et al. (2007), using *t*-tests, identified the

decreased kidney weights in the 33% MON 863 males as statistically significant from the near-isogenic control males. In any case, the Panel noted the differences between male treated and control kidney weights is small (*i.e.*, <10%), and the mean of the 33% males for the kidney weight observations does not differ from the 6 reference groups. The absence of changes in blood urea nitrogen and creatinine further indicates the lack of an adverse effect of MON 863 on renal function.

6.4. Pathology data

Post-mortem examination of selected tissues from the high-dose (33%) MON 863 treated groups and the respective high-dose control animals revealed only one statistically significant difference in the incidence of microscopic findings (Hammond et al., 2006).

The incidence of renal tubular mineralization was found to be significantly lower in MON 863 treated females than in the control females (*i.e.*, 2/20 *versus* 9/20). Even if this were regarded as a treatment-related effect, it would not be considered adverse. Other differences in the incidence of microscopic findings were observed between the MON 863 high-dose and control animals; however, none were statistically significant.

As stated earlier, the kidney tissues were subjected to a re-examination by a Pathology Working Group (PWG). The PWG also reviewed clinical and organ weight data relevant to kidney function. They concluded that there was no evidence that MON 863 grain had any adverse effects on rat kidney function (see Section 1).

7. Discussion of the overall biological importance of Séralini et al. (2007) findings

The Panel reviewed and assessed the Séralini et al. (2007) reanalysis of the 90-day rat oral toxicity study on MON 863 corn (Covance, 2002) both in terms of the statistical methods utilized and with respect to the biological importance of the reported findings. In turn, the significance of the results reported by Séralini et al. (2007) in relation to the original analysis reported in Hammond et al. (2006) was evaluated.

First, prior to a discussion of the statistical and biological importance of the findings reported by Séralini et al. (2007) and Monsanto (Hammond et al., 2006), the Panel has noted errors of fact presented in the Séralini et al. (2007) report. Séralini et al. (2007) state that the Cry protein is toxic to human cells and that Cry3Bb1 has not been adequately tested. This is not the case. The Cry3Bb1 protein had been tested in a high-dose acute toxicity study in rats, as required by US EPA for registration of insect protected crops (Hammond et al., 2006). Moreover, the Cry proteins introduced into biotech crops are not toxic to mammals (Betz et al., 2000). The articles cited by Séralini et al. (2007) to support the contention that Cry proteins were toxic in fact mostly relate to other proteins, the Cyt toxins (Federici, 2002), produced by certain strains of *B. thuringiensis* (*Bt*). The Cyt proteins are known to be toxic to invertebrate and vertebrate cells. MON 863 does not contain genes encoding for these types of proteins.

Secondly, in the original study, 6 reference groups were treated with other non-GMO corn varieties to provide additional contemporaneous data from which to compare the MON 863 and near-isogenic parental strain treatment groups. Data from the 6 reference corn varieties provide valuable information pertaining to the range of responses that could be expected from rats treated for 90 days with diets containing 33% corn. Séralini et al. (2007) discarded all of the data from the reference groups at least in part based on their assertion that diets of these groups were not of the same composition. A review of the diets prepared demonstrates this is not the case (see Table 1). The diets, with respect to key nutritional parameters including calorie fat and protein content, *etc.* were comparable, thus, it is appropriate to use the reference groups to assist in the interpretation of the pair-wise comparisons between the 11% and 33% MON 863 groups and the respective near-isogenic controls.

With respect to the statistical analyses, it should be noted that Séralini et al. (2007) repeated the statistical analysis conducted in the Monsanto analyses by Hammond et al. (2006) and obtained the same results with regard to the descriptive statistics.

The statistical analyses of the serum biochemistry, haematological, and clinical chemistry data conducted by Séralini 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 Séralini 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. The principle difference between the Monsanto and Séralini et al. (2007) analyses was in the evaluation of the body weight data. Monsanto used 'traditional' ANOVA and parametric analyses while Séralini 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 Séralini et al. (2007) accounted for the changing variance and the correlated nature of the body weight data over time.

The Panel has evaluated the biological importance (*i.e.*, the relevance to the safety assessment of MON 863 corn) of the statistical findings reported in Séralini et al. (2007). As noted previously, the biological importance of any given statistical finding is determined through assessment of dose–response, reproducibility over time, association with other relevant changes (*e.g.*, histopathology), occurrence in both sexes, magnitude of response, knowledge regarding

the normal variation of response in the experimental animals used in the study (*i.e.*, historical and contemporaneous controls), biological plausibility, and other factors.

The finding by Séralini et al. (2007) of statistical significance associated with lower body weights in 11% MON 863 males (496 g *versus* 513 g for the respective control) is of no biological importance since the finding is small (~−3%), was not replicated in females, and did not occur at the higher dose level in the 33% males. In the Monsanto analysis (Hammond et al., 2006), the mean of the 11% and 33% MON 863 treated groups was not significantly different from the mean of the 6 reference controls. Given this and the bi-directional nature of the reported findings of Séralini et al. (2007) (*i.e.*, increased body weight in high-dose females and decreased body weight in 11% males), the lack of biological plausibility of the possible cause proposed by Séralini et al. (2007) (*i.e.*, organ and/or hormonal dysfunction), the lack of dose–response, and the small difference, the body weight findings are considered to be unrelated to MON 863 treatment and probably the result of normal variation.

A number of haematological, clinical chemistry, and urinalysis values were reported by Séralini et al. (2007) to be significantly different from the respective controls. To aid in the interpretation of these results comparisons to the 6 reference groups fed different corn varieties can be made. The use of contemporaneous reference groups, as used in the Hammond et al. (2006) study is a commonly accepted practice to assist in the interpretation of statistically significant values (Task Force of Past Presidents, 1982; Deschl et al., 2002). Moreover, interpretation of the biological importance of observed statistically significant changes in haematological and clinical chemistry endpoints requires careful examination of dose– and temporal–response relationships, and of how individual endpoints associated with various organ functions (*e.g.*, serum BUN and creatinine, and urinary volume, protein and specific gravity with respect to kidney function) differ between control and treated groups. Isolated changes in a single variable in the absence of changes in other related variables, especially in the absence of histopathological effects, are probably not treatment-related effects (Wilson et al., 2001; Lewis et al., 2002).

Overall, given that the statistically significant findings, either reported by Séralini et al. (2007) or Monsanto (Covance, 2002; Hammond et al., 2006), displayed one or more of the following traits: (a) small changes, (b) no temporal (week 5 *versus* week 14 bleed) or dose–response relationship (33% *versus* 11%), (c) occurrence in only one sex, and (d) lack of change in correlated parameters, which, together with no correlative histopathological findings in haematopoietic or lymphoid tissues, are considered to be of no clinical or biological importance.

The finding of smaller absolute and relative kidney weights in the high-dose males (~−7% *versus* the respective near-isogenic control group) is also considered of no biological importance as the mean of the kidney weights of the 33% MON 863 males were not different from the reference groups mean and was not associated with any histopathological changes (Hard and Terron, 2004) or any alterations in clinical chemistry or urinalysis parameters indicative of compromised kidney function.

8. Conclusions

The Panel concludes that the original analysis conducted by Monsanto (Hammond et al., 2006) used statistical methods conventionally recognized by regulatory authorities (EFSA, 2004). The methods used in the reanalysis conducted by Séralini et al. (2007) for clinical chemistry, haematological and urinalysis variables, were broadly comparable in concept with the original analysis conducted by Monsanto (Hammond et al., 2006), except that Séralini et al. (2007) made comparisons between groups using *t*-tests without multiple comparison procedures and without an initial one-way ANOVA. The differences in the two approaches probably led to the higher rate of statistically significant comparisons in the Séralini et al. (2007) analysis compared to the Monsanto analysis.

With respect to the body weight analysis, Monsanto used traditional parametric statistical analysis and visualization of growth curves, while Séralini et al. (2007) used an approach based upon the Gompertz model to estimate the body weight data over time. The lack of details in their paper regarding the handling of the correlation of the repeated body weight measures over time and of the assumptions regarding increased variation in body weight as the study progressed limits the interpretation of the reported results. The Gompertz model has rarely been used to assess body weight data, and, as a result, it is not possible to evaluate the significance of the results reported relative to historical evaluations that have used parametric tests to evaluate the effects of test substances on animal body weight.

The Panel concludes that the Séralini 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 Séralini 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.

The conclusions of the Panel are consistent with the recently announced conclusions of the European (EFSA, 2007b), German (Bfr, 2007), French (AFFSA, 2007), and Philippine (Yap, 2007) regulatory authorities. EFSA (2007b), Bfr (2007), AFFSA (2007), and Yap (2007) all concluded that the Séralini et al. (2007) paper does not

advance any new scientific data to indicate that MON 863 caused adverse effects in the 90-day rat study.

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References

AFFSA, 2007. de l'Agence française de sécurité sanitaire des aliments relatif à la récente étude publiée1 sur le maïs génétiquement modifié MON 863. Agence Française de Sécurité Sanitaire Des Aliments. La Directice Générale. AFFSA Dossier No. 2007-SA-0109, 26 April 2007.

Betz, F., Hammond, B.G., Fuchs, R.L., 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. Regul. Toxicol. Pharmacol. 32, 156.

Bfr, 2007. 90-Tage-Studie an Ratten mit MON 863-Mais: Keine Hinweise auf gesundheitliches = [Risiko90-Day Study on rats with MON 863 Maize: No Indication of a Health Risk]. Bundesinstitut für Risikobewertung (BfR) [Federal Institute for Risk Assessment], Berlin. Opinion No. 009/2207 from the BfR on 29 March 2007. <http://www.BfR.bund.de/cm/208/90_tage_studie_an_ratten_mit_mon863_mais.pdf>.

CGB, 2004. Compte rendu synthetique de la séance du 23 novembre 2004 (MON 863) = [CGB opinion of 23 November 2004 on MON 863]. Commission du genie biomoléculaire (CGB). <http://www.ogm.gouv.fr/experimentations/evaluation_scientifique/cgb/CR_seances/CRsynth0411-23.pdf>.

Chan, P.K., O'Hara, G.P., Hayes, A.W., 1982. Principles and methods for acute and subchronic toxicity. In: Hayes, A.W. (Ed.), Principles and Methods of Toxicology. Raven Press, New York, pp. 1–51.

Cockburn, A., 2003. Assuring the safety of genetically modified (GM) foods: the importance of a holistic, integrative approach. J. Biotechnol. 98, 79–106.

Covance, 2002. 13-Week Dietary Subchronic Comparison Study with MON 863 Corn in Rats Preceded by a 1-Week Baseline Food Consumption Determination with PMI Certified Rodent Diet #5002. Prepared by: Covance Laboratories, Inc., Vienna, Virginia for Monsanto Company, St. Louis, Missouri. Covance Study No. 6103-293, 17 December 2002.

Crawley, M.J., 2005. Statistics: An Introduction Using R. Wiley Publishers, London (Cited in Séralini et al., 2007).

Deschl, U., Kittel, B., Rittinghausen, S., Morawietz, G., Kohler, M., Mohr, U., Keenan, C., 2002. The value of historical control data-scientific advantages for pathologists, industry and agencies. Toxicol. Pathol. 30, 80–87.

EC, 2003. Genetically Modified Crops in the EU: Food Safety Assessment, Regulation, and Public Concerns. The European Network on Safety Assessment of Genetically Modified Crops (2000–2003). European Commission, Directorate-General for Research Biotechnology, Agriculture and Food Research, Brussels.

EFSA, 2004. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from insect-protected genetically modified maize MON 863 and MON 863 × MON 810, for which a request for placing on the market was submitted under Article 4 of the Novel Food regulation (EC) No. 258/97 by Monsanto. EFSA J. 49, 1–24. <http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_opinions/383.Par.0001.File.dat/opinion_gmo_07_en1.pdf>.

EFSA, 2006. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. EFSA J. 99, 1–94, 2004 (Final edited version May, 2006). <http://www.efsa.eu.int/cf/consultation.cfm>.

EFSA, 2007a. Statement of the GMO Panel on the Safe Use of the *npt*II Antibiotic Resistance Marker Gene in Genetically Modified Plants. European Food Safety Authority (ESFA). <http://www.efsa.europa.eu/en/science/gmo/statements0/npt2.html>.

EFSA, 2007b. Statement of the Scientific Panel on Genetically Modified Organisms on the analysis of data from a 90-day rat feeding study with MON 863 maize. European Food Safety Authority (ESFA). Adopted 25 June 2007. <http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/statements.Par.0001.File.dat/GMO_statement_MON863.pdf>.

FDA, 2000. Toxicological Principles for the Safety Assessment of Food Ingredients: Redbook 2000. Food and Drug Administration, US (FDA), Center for Food Safety and Applied Nutrition (CFSAN), Washington, DC.

Federici, B., 2002. Case study: *Bt* crops. In: Atherton, K. (Ed.), Genetically Modified Crops, Assessing Safety. Taylor and Francis, London.

FAO/WHO, 1996. Biotechnology and Food Safety. Report of a Joint FAO/WHO Consultation, September 30–October 4, 1996, Rome, Italy. Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO). FAO Food and Nutrition Paper 61.

FAO/WHO, 2000. Safety Aspects of Genetically Modified Foods of Plant Origin. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, May 29–June 2, 2000, Geneva, Switzerland. Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO).

FAO/WHO, 2003. Report of the Fourth Session of the Codex *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology, March 11–14, 2003, Yokohama, Japan to the Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Twenty-sixth Session, June 30–July 7, 2003, Rome, Italy. Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) [Alinorm 03/34A]. <http://www.codexalimentarius.net/web/archives.jsp?year=03>.

Gad, S.C., 1998. Statistics and Experimental Design for Toxicologists. CRC Press, Boca Raton, FL (Cited in Gad, 2001).

Gad, S.C., 2001. Statistics for toxicologists. In: Hayes, A.W. (Ed.), Principles and Methods of Toxicology, fourth ed. Taylor and Francis, Philadelphia, pp. 285–364.

George, C., Ridley, W.P., Obert, J.C., Nemeth, M.A., Breeze, M.L., Astwood, J.D., 2004. Composition of grain and forage from corn rootworm-protected corn event MON 863 is equivalent to that of conventional corn (*Zea mays* L.). J. Agric. Food Chem. 52, 4149–4158.

Hammond, B., Ward, D., 2004. Supplemental Analysis of Selected Findings on the Rat 90-Day Feeding Study with MON 863 Maize Report MSL-18175. Submitted to the French CGB by Monsanto (Unpublished report).

Hammond, B., Lemen, J., Dudek, R., Ward, D., Jiang, C., Nemeth, M., Burns, J., 2006. Results of a 90-day safety assurance study with rats fed grain from corn rootworm-protected corn. Food Chem. Toxicol. 44, 147–160.

Hard, G., Terron, A., 2004. Pathology Peer Review, 003-RS-04. Retrospective Evaluation of Renal Tissues and data from Monsanto Company Study CV-200-260 (MSL 18175): A 13-Week Rat Feeding Study with MON 863 corn. Monsanto Company (Unpublished report).

Hattan, D., 1996. Evaluation of toxicological studies on Flavr Savr tomato. Food Safety Evaluation. Organisation for Economic Co-operation and Development (OECD), Paris, pp. 58–60.

ILSI, 2004. Nutritional and Safety Assessments of Foods and feeds Nutritionally Improved through Biotechnology. Prepared by a Task Force of the ILSI International Food Biotechnology Committee. International Life Sciences Institute (ILSI)/Institute of Food Technologists (IFT). IFT Comprehensive Reviews in Food Science and Food Safety, vol. 3.

Kuiper, H.A., Kleter, G.A., Kok, E.J., 2001. Assessment of the food safety issues related to genetically modified foods. Plant J. 27, 503–528.

Kuiper, H.A., Kleter, G.A., Hub, P.J.M., Kok, E.J., 2002. Substantial equivalence – an appropriate paradigm for the safety assessment of genetically modified foods. Toxicology 181–182, 427–431.

Lewis, L.W., Billington, R., Debryune, E., Gamer, A., Lang, B., Carpanini, F., 2002. Recognition of adverse and non-adverse effects in toxicity studies. Toxicol. Pathol. 30, 66–74.

LSRO, 1998. Alternative and Traditional Models for Safety Evaluation of Food Ingredients. Prepared by American Society for Nutritional Sciences, Life Sciences Research Office (LSRO), Bethesda, Maryland for Center of Food Safety and Nutrition (CFSAN), Food and Drug Administration, US (FDA), Washington, DC (FDA Contract No. 223-92-2185).

MacKenzie, D., 2000. International Comparison of Regulatory Frameworks for Food Products of Biotechnology. Prepared for the Canadian Biotechnology Advisory Committee Project Steering Committee on the Regulation of Genetically Modified Foods.

McClintock, J.T., Schaffer, C.R., Sjoblad, R.D., 1995. A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. Pest. Sci. 45, 95–105.

Munro, I.C., McGirr, L.G., Nestmann, E.R., Kille, J.W., 1996. Alternative approaches to the safety assessment of macronutrient substitutes. Regul. Toxicol. Pharmacol. 23, S6–S14.

OECD, 1993. Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles. Organization for Economic Cooperation and Development (OECD), Paris.

OECD, 1998. Repeated Dose 90-Day Oral Toxicity Study in Rodents. OECD Guideline for the Testing of Chemicals. Guideline 408. Adopted 21 September 1998. Organization for Economic Cooperation and Development (OECD), Paris.

OECD, 2002a. Task Force for the Safety of Novel Foods and Feeds. Sixth Session of the Task Force on the Safety of Novel Foods and Feeds, Chateau de la Muette, 17–19 June 2002. Organization for Economic Cooperation and Development (OECD), Paris, ENV/JM/FOOD(2001)8/REV1.

OECD, 2002b. Environmental Health and Safety Publications. Series on Testing and Assessment No. 35 and Series on Pesticides No. 14. Guidance Notes for Analysis and Evaluation of Chronic toxicity and Carcinogenicity Studies. Organization for Economic Cooperation and Development (OECD), Paris, ENV/JM/MONO(2002)19, 4 September 2002, pp. 37–38.

Ridley, W.P., Nemeth, M.A., Astwood, J.D., Breeze, M.L., Sorbet, R., 2002. Amended Report for MSL-17199: Compositional Analyses of Forage and Grain Collected from Corn Rootworm Protected Maize Event MON 863 Grown in 1998 US Field Trials. Monsanto Company (Unpublished report MSL-17669).

RKI, 2003. Assessment Report of the Robert Koch Institut in Accordance with Directive 2001/18/EC, 8 April. 2003. Insect Resistant Maize MON 863 and MON 863 X MON 810. Application by Monsanto Company, USA, Represented by Monsanto Europe SA, Brussel, Belgium, for the Placing on the Market of Genetically Modified Maize (Draft Translation of Application C/DE/02/9). Robert Koch Institut, Berlin. <http://www.transgen.de/pdf/zulassung/Mais/MON863_MON863xMON810_assessment.pdf>.

SAS, 1999. SAS®, Version 8 On-line Documentation, 1999. SAS Institute, Inc., Cary, North Carolina (Cited in Hammond et al., 2006).

Séralini, G.-E., Cellier, D., de Vendomois, J.P., 2007. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Arch. Environ. Contam. Toxicol. 52, 596–602.

Siegel, J.P., 2001. The mammalian safety of *Bacillus thuringiensis*-based insecticides. J. Invertebr. Pathol. 77, 13–21.

Sjoblad, R.D., McClintock, J.T., Engler, R., 1992. Toxicological considerations for protein components of biological pesticide products. Regul. Toxicol. Pharmacol. 15, 3–9.

SOT, 1989. Continuing Education Course #8 Statistics for Toxicologists. Society of Toxicology (SOT), 28th Annual Meeting, February 27, 1989, Atlanta, Georgia.

Task Force of Past Presidents, 1982. Animal data in hazard evaluation: paths and pitfalls. Fundam. Appl. Toxicol. 2, 101–107.

Taylor, M.L., Hyun, Y., Hartnell, G.F., Riordan, S.G., Nemeth, M.A., Karunanandaa, K., George, B., Astwood, J.D., 2003. Comparison of broiler performance when fed diets containing grain from YieldGard Rootworm (MON 863), YieldGard Plus (MON 810 × MON 863), nontransgenic control, or commercial reference corn hybrids. Poultry Sci. 82, 1948–1956.

US EPA, 1998. Reregistration Eligibility Decision (RED) *Bacillus thuringiensis.* US Environmental Protection Agency (US EPA), Prevention, Pesticides and Toxic Substances (7508W), Washington, DC, EPA738-R-98-004. <http://www.epa.gov/oppsrrd1/REDs/0247.pdf>.

US EPA, 2004. *Bacillus thuringiensis* Cry3Bb1: exemption from the requirement of a tolerance: final rule (US Environmental Protection Agency). Fed. Regist. (US) 69, 16809–16814.

Weil, C.S., 1962. Application of methods of statistical analysis to efficient repeated-dose toxicological tests: I. General considerations and problems involved sex differences in rat liver and kidney weights. Toxicol. Appl. Pharmacol. 4, 561–571.

Weil, C.S., 1973. Experimental design and interpretation of data from prolonged toxicity tests. In: Proceedings, Fifth International Congress of Pharmacology, vol. 2. Beacon Press, San Francisco, pp. 4–12 (Cited in Gad, 2001).

Weil, C.S., 1982. Statistical analysis and normality of selected hematologic and clinical chemistry measurements used in toxicologic studies. Arch. Toxicol. Suppl. 5, 237–253.

Weil, C.S., Gad, S.C., 1980. Applications of methods of statistical analysis to efficient repeated-dose toxicologic tests: 2. Methods for analysis of body, liver, and kidney weight data. Toxicol. Appl. Pharmacol. 52, 214–226.

WHO, 1987. Principles for the Safety Assessment of Food Additives and Contaminants in Food. World Health Organization (WHO), International Programme on Chemical Safety (IPCS), Geneva, Switz. Environmental Health Criteria, No. 70. <http://www.inchem.org/documents/ehc/ehc/ehc70.htm>.

WHO, 1999. Microbial Pest Control Agent: *Bacillus thuringiensis*. World Health Organization (WHO), International Programme on Chemical Safety (IPCS), Geneva. Environmental Health Criteria, No. 217. <http://www.inchem.org/documents/ehc/ehc/ehc217.htm>.

Williams, D.A., 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics 27, 103–117 (Cited in Gad, 2001).

Williams, D.A., 1972. The comparison of several dose levels with a zero dose control. Biometrics 28, 519–531 (Cited in Gad, 2001).

Wilson, N.H., Hardisty, J.F., Hayes, J.R., 2001. Short-term, subchronic, and chronic toxicology studies. In: Hayes, A.W. (Ed.), Principles and Methods of Toxicology, fourth ed. Taylor and Francis, Philadelphia, pp. 917–957.

Yap, A.C., 2007. Review of the Issue Regarding the Rat Feeding Study of GM Corn Event MON 863. Memorandum for the President from Secretary Arthur C. Yap. Republic of the Philippines, Department of Agriculture, May 16, 2007.