

Evaluation of and Final Report on the summary report of the "13-Week Dietary Subchronic Comparison Study with MON 863 in Rats Preceded by a 1-Week Baseline Food Consumption Determination with PMI Certified Diet #5002 (Report MSL-18175/Covance Study No. 6103-293)".

By Arpad Pusztai, 15 September 2004

This report only deals with the results of the MON 863 feeding study. Although some of the results of other studies with MON 863 are not confidential and thus have been available to all, my comments will still strictly be confined to the feeding study.

General comment:

The design of the feeding study is not well focussed, with many flaws and crucial omissions in it and not up to date of what is expected of such an important study. The experiments are poorly executed in many instances and the presentation of the results is fragmentary, repetitive, not well set out and confusing. Although the results are tabulated in big Tables, the content of these is generally uninformative. There is a lot of superfluous data presented taking up a great deal of space but without making any significant contribution to our understanding. The use of historic values and the comparisons of the test and parental control diets with an additional six reference diet groups may have some relevance in commercial production studies but not in a scientific risk analysis where the comparison must be between the GM corn diet and the corresponding control diets (see later!). The inclusion of these additional reference groups only serves to widen the range of the data in the statistical analyses and thus to reduce the chances of finding significant differences between the test and control groups. In any case, these so-called reference groups are only selectively used in the comparisons when this serves the purpose of the authors. I shall point and set out these in my detailed comments below.

Detailed comments:

Diet composition, formulation and other relevant problems:

It is uninformative and unacceptable to describe the preparation of the diets as done "according to specifications" even if some aspects of the composition are apparently confirmed by analysis. For example, MON 863 was reported to contain 11.3% protein while the control line only 9.9%. This was in addition to other compositional differences such as fibre, etc. However, in the diets the protein content and other ingredients were equalized but we were not told where did the extra 12-13% protein, etc. come from in the control diets. Nothing is given about the equalization and optimization of the essential amino acid (e.g. lysine, etc) content of the diets, either. As the diets were apparently stored at room temperature for the duration of the study we are not told whether the composition of the diet remained the same throughout or

not and whether this was checked or not. Many sensitive ingredients in the diets could have been oxidized or otherwise changed to influence the nutritional value of the diets. There is only a reference to a gravimetric record of dietary mixing on p. 19 and apparently salt analysis was used as a surrogate for homogeneity testing! The precise composition of the diets is on file with the sponsor (p. 17).

It is unclear why the 11% test diet was not supplemented with the parental line instead of commercial maize. In any case, it is possible that in the USA the commercial maize samples are already contaminated by GM maize, such as the glyphosate-resistant NK 603.

A major omission is that, in addition to the parental line control group, the authors should have used another proper control group in which the parental line diet was supplemented with the transgene product isolated from MON 863 maize at the same concentration as it is expressed in MON 863. This should have made it possible to show up any effects due to the splicing of the Bt gene construct into the corn genome.

Animal procedures:

Unfortunately both the design and the execution of the feeding study was poor.

For reasons that are not clear at the beginning of the report the starting weight of the rats is given as 198.4 to 259.8 g for males and 132.1 to 185.3 g for females, all claimed to be within $\pm 2SD$. However, in Appendix 2 (individual body weight data, starting at p. 161) the values are given as 143 to 186 g for males and 100 to 169 g for females.

In the results it is stated that there were no significant differences between the test and control groups in the final weight of the rats, their growth and food consumption. However, these were mean values with considerable SD values. Moreover, the range of the values considerably widened during the experiment even though the feed intake of the rats was reasonably similar. Thus, weight accretion during the experiment varied between 265 to 370 g for males and 110 to 156 g for females. Moreover, rats with the highest starting weight occasionally ended up with the smallest final weight. The most likely explanation for these erratic results is poor animal management. Unfortunately under such conditions it is very difficult to make proper comparisons between the groups because it is difficult to know the reason for the differences in the results. Thus, claims that this particular GM maize had no significant effect on rat growth are not supported by the data.

There were further problems with the growth of the rats. The feed intake of the rats was fairly similar throughout the experiment. However, the growth was uneven. By week 7 body weight changes became very erratic and in the last four weeks the rats hardly grew. This meant that food conversion ratio dropped catastrophically in the last few weeks of the experiment. No explanation was given. In my opinion the most likely explanation for this, apart from mismanagement of the animals, is that there were probably problems with the nutrient composition of the diets, possibly due to the inclusion of maize in them. However, as no relevant and precise information is given in the submission about what actual proteins were included in the diet to make up their total protein content, nothing further can be said about it.

In some weeks in some of the animals body weight changes were negative which were then followed by unusually large positive changes. For example, male rat no. 38612 dropped 53 g in week 11 but then gained 102 g in week 12. These problems again indicate poor animal management, questioning the value of the work and making it difficult to draw any meaningful conclusions.

Observation of the animals

Although a number of important organs are weighed (wet but not dry weights), including the liver, kidneys, etc. no part of the gastrointestinal tract or any of the muscles are weighed to establish whether the GM maize diet did have any effect on them despite the fact that there are many papers in the literature that indicate such effects.

Clinical Pathology

Most of the measurements are mechanistic, conservative and static. Although the results could be used as a starting point for further more dynamic investigations but without following up the observed changes in the animals on GM diet the only thing what we are left with is the possibility of debating the significance or non-significance of the findings. For example, increased lymphocyte counts could mean problems with the immune system such as infections, etc. However, the authors never measured the immune responsiveness of the rats or the levels of specific humoral or mucosal antibodies to components of the GM maize and particularly to the expressed Bt toxin even though that there are published reports in high-class journals that this could occur. It is also known that changes in basophil counts could signify changes in allergenicity and IgE levels. Even though this is a potential major concern with GM diets no attempts were made to follow it up. And one can go on!

Significant haematology effects:

General comment. There were many significant differences between the blood constituents of the 33% GM maize diet-fed rats and the REF controls. However, the possible significance of these is underplayed by the authors in this case

MALES:

There are significant differences in WBC, lymphocyte counts, basophil counts and APPT between rats on 33% GM maize diet vs. control

There are also significant differences in RBC, haemoglobin, haematocrit (not fully), MCHC, WBC, reticulocytes, lymphocytes, basophils between rats on GM maize diet vs. REF controls.

FEMALES:

RBC, haemoglobin, reticulocytes (at both weeks 5 and 14), basophil counts were significantly different in GM maize-fed rats vs control.

MCHC, reticulocytes, basophil counts, prothrombin time and APPT in GM maize-fed rats were all significantly different from those in REF controls.

Blood chemistry:

MALES

Protein, albumin, globulin, alanine amino transferase, calcium, chloride, glucose and creatinine were different in GM maize-fed rats from control

Albumin, alkaline phosphatase, inorganic phosphate, urea were different in GM maize-fed rats vs. REF controls.

FEMALES

Albumin, globulin, cholesterol, triglycerides were different in GM maize-fed rats vs. control.

Triglycerides, alanine amino transferase, calcium, inorganic phosphorus were different in rats given GM maize vs. REF controls.

Urine Chemistry has also shown up many significant differences between GM-fed rats and controls.

Anatomic Pathology - Necropsy

The description of what was done is incredibly inadequate. Apparently what was done is that trained personnel using procedures approved by board-certified pathologists examined, *eye-balled*, of the carcass, body orifices, abdominal, thoracic and cranial cavities and organs/tissues. What follows is summary Tables of clinical and macroscopic observations (Tables 1,3,4,5), page-after-page of almost meaningless padding. The only purpose of all this is to tire out the reader by filling him up with numbers but without providing them with any information. It is all the more remarkable that if one keeps reading eventually in Table 6 one gets some, albeit qualitative, information indicating that the liver, kidneys, stomach and rectum in male rats (somewhat similar in females) fed the 33% GM maize diet are more affected than the corresponding controls.

Tissue preservation - Histopathology

The information given out on this is that formalin-preserved tissues are embedded in paraffin, sectioned, stained with haematoxylin and eosin and then examined microscopically. Very little is revealed about the methodologies used in the study.

Conclusions:

Although this imperfectly designed and executed study has revealed a huge list of significant differences between the various biologically meaningful parameters of rats fed GM maize diets and the proper controls or even the REF controls, it would be impossible for anyone to state that all these statistically significant differences are also biologically significant. However, the opposite cannot be said either without proper follow up studies. Some examples and suggestions were given in this critical appraisal.

First and foremost, a more modest but properly designed and better controlled and executed experiment would have given us more confidence in the validity of all the various experimental values and the comparability of the data of the various experimental groups. As it is, the whole experiment will have to be repeated. However, the list of significant differences suggest that the authors' confidence that the genetic modification of the maize sample has induced no significant changes in the nutritional value and the biological/immunological, etc. properties of this important food/feed crop is almost certainly groundless. It is almost impossible to imagine that major lesions in important organs (kidneys, liver, etc) or changes in blood parameters (lymphocytes, granulocytes, glucose, etc) that occurred in GM maize-fed rats, is incidental and due to simple biological variability. There is an urgent need to move away from simple mechanistic analytical work that has no hope of describing the dynamic situation that occurs on feeding GM maize.

It is a pity that so much work has brought so little dividend. With more critical attention to the nutritional/toxicological/immunological works that had been done and published with GM crops the authors could have made a real contribution to our understanding of the effects that GM foods can have on humans and all other important animal species.

Recommended reading: Pusztai et al. (2003) "Genetically Modified Foods: Potential Human Health Effects" in Food Safety: Contaminants and Toxins (ed. By JPF D'Mello), CABI Publishing, Wallingford, Oxon, UK, pp. 347-372. ISBN 0 85199 607 8