Monsanto's GE corn: Unfit for rats, unfit for humans

Greenpeace's assessment of Monsanto's study "Supplemental analysis of selected findings on the rat 90-day feeding study with MON863 maize. Report MSL-18175."

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Summary

Monsanto have provided additional detail on a 90-day rat feeding study with the GE (genetically engineered) Bt Corn, MON863. This study demonstrates that there are many irregularities in the study and significant differences between the high dose and control groups. These include:

1) Lack of relevant information to allow verification
2) Inappropriate use of "reference" ranges and "historical" values
3) Countering statistically valid differences
4) Statistically significant difference in white cell counts
5) Statistically significant difference in lymphocyte counts
6) Statistically significant difference in reticulocyte counts
7) Statistically significant differences in serum glucose levels
8) Statistically significant difference in kidney weights
9) Different renal tubular mineralization

Monsanto has tried to refute these 5 statistically valid differences by using "reference" and "historical" values, which have not formed part of the experimental study. The inclusion of these "reference" and "historical" values is not appropriate as the critical comparison is between the GE maize fed animals and the experimental controls.

Disturbingly, Monsanto has also interpreted the significant results as "not of biological significance" and "statistical aberrations". The feeding trial is over a short period of time, 90 days. If the application to market this GE crop is successful, exposure of humans and animals to MON863 is likely to be of much longer duration than 90 days. Therefore, whilst this 90-day feeding trial can not assess the safety of the GE food, it can give warning signals of adverse effects. As this study is a major part of the evidence presented for food safety, it is vital that valid conclusions are drawn from statistically rigorous comparisons between the control and dose groups. To disregard the findings of this study could be a danger to human and animal health.

The high number of statistically significant differences between rats fed high dose MON863 and the controls in this short feeding trial should be cause for concern and MON863 should be rejected outright.

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What was done?

This study from Monsanto details the further details on subchronic toxicity data for rats fed MON863 requested by the French Competent Authorities. MON863 is a GE Bt corn, which expresses the Cry3Bb1 protein. However, it is different to the more common GE Bt (Cry1Ab) corn, as a different Bt protein is produced The target organisms for the protein in MON863 (Cry3Bb1) is corn rootworm (Coleopteran (beetles and weevils), *Diabrotica sp.*), whereas the Cry1Ab protein (as produced by, e.g. MON810, Bt11, Bt176) is toxic to the European corn borer (Lepidopteran (butterflies and moths), *Ostrinia nubilalis*).

According to the study, 10 groups of 20 genetically standardised rats (specially bred for this type of testing) were fed feed containing 33 % maize. Four trials were conducted, allowing two comparisons were made 1) Comparison between rats fed no GM maize and a low dose MON863 (11 % of the feed, with another 22 % from conventional maize) and 2) Comparison between rat4s fed no GM maize and a high dose MON863 (33 % of the feed, with no conventional maize). Separate control groups were used for each comparison (low - 11 % and high – 33 %).

What's wrong with the study?

1) Lack of relevant information to allow verification

In the data tables, only ranges are presented, no standard deviation nor number of samples are given. Both are normally given in the scientific literature. Although the number of samples can be inferred be the same as given in the method, the lack of standard deviation data makes independent verification of the significant differences impossible.

2) Inappropriate use of "reference" ranges and "historical" values

Monsanto places considerable emphasis on "reference" ranges (additional reference control groups) for the parameters measured. Their use here as a "normal" range is invalid because the critical comparison is between the doses and respective control groups included in the study. To compare means to ranges of additional groups is statistically meaningless. The use of "historical" values is similarly invalid. The OECD protocol ensures that the study is performed under the same conditions to both dose and control groups so that statistically valid comparisons can be made. The use of these normal or "reference" ranges has the effect of obscuring statistically valid differences. These significant differences could be indicative of adverse effects and therefore warrant further and full investigation.

3) Countering statistically valid differences

At several points in the study, Monsanto states that statistically differences can be false positives, either arrived at by chance, or through natural biological variation. It is true that statistically significant analysis can lead to false positives (also false negatives) but these can be **indicative** of adverse effects and should therefore be investigated fully. This is particularly important as the feeding trial is only 90 days. There are no longer term experiments. Therefore, any possible differences in this short-term study must be investigated further as the study forms a major part of the food "safety" assessment of the GE maize.

4) Statistically significant difference in white cell counts

Average white cell counts were significantly different (at the 95 % confidence interval) between the control rats and the high dose (33 % MON863) male rats. White cell counts were increased by 20.4 %

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Monsanto says: "*All but one of the high dose individual male white blood cell counts is within the range of reference control values. Graphic presentation of this [sic.] data indicate there is a minimal shift in MON863 white blood values compared to other control group values. However, this shift is nearly identical to the apparent downward shift observed in reference control group 8 values. Moreover, at week 5, the white blood count in MON863 (which was not different than parental controls), was higher than them at week 14. These data sets are an example of normal biological variability. Furthermore, there is no dose related effect observed in female white cell count.*"

Greenpeace says: The fact that one value lies outside the reference range confirms the statistical significance of this finding. No data are presented over the 90-day time period. The study is recommended for 90 days and that is the timeframe for reporting. It is irrelevant to cite variation over the time of a standard test. Such a study is recommended by the OECD to be conducted over 90 days. This allows sufficient time for any differences to emerge clearly. It is not only impossible, but also dangerous to assume these results are solely the result of "normal biological variability". A significant difference has been found and should be investigated fully. The relevance of no dose related effect in females is highly questionable. The different responses between male and female rats should not be used to add weight to the "normal biological variability". It is quite possible that males and females react differently and that only one gender may be adversely affected. Does this study recommend that only females should eat MON863?

5) Statistically significant difference in lymphocyte counts

Similarly, lymphocyte counts were significantly different (at the 95 % confidence interval) between the control rats and the high dose (33 % MON863) male rats. White cell counts were increased by 22.1 %. The increase in white blood cell count is attributed to this increased lymphocyte count.

Monsanto says: "*All of the high dose individual male lymphocyte vales fall within the range of values measured for that reference control group and the male high dose mean is lower than that of the historical mean of 9.2 for this strain of rat utilized in other studies conducted by Covance Laboratories [who performed the study].*"

Greenpeace says: To disregard this finding on the basis of the reference group range and historical mean is invalid. The critical comparison is between the high dose rats and control group. Why else does the experimental protocol require the use of controls?

6) Statistically significant difference in reticulocyte counts

A statistically significant difference was observed in reticulocyte counts between high dose (33 % MON863) and control in female rats. The MON863 fed rat had 51.8 % lower reticulocyte counts than the control.

Monsanto says: "*The 34 % and 52 % decrease in reticulocyte counts in the low and high dose females, respectively, are attributed to normal biological variability. With the exception of one animal each in the low and high dose groups with a reticulocyte count of 0.01, all MON863-fed female reticulocyte values fall well within the range of reference control values.*"

Greenpeace says: Once again, the fact that the ranges extend beyond the reference range confirms the statistical significance of this finding. The high dose group shows clear and marked significant differences to the control group. This finding should be investigated thoroughly.

7) Statistically significant differences in serum glucose levels
Statistically significant differences in serum glucose levels were observed in both the low dose (11 % MON863) and high dose (33 % MON863) female rats. Serum glucose levels were elevated by 9 and 10 % (low and high doses respectively).

Monsanto says: "*The 9 % and 10 % increases in serum glucose observed in low and high dose females, respectively, are of no biological consequence and very unlikely attributable to MON863 exposure. The range of MON863 individual animal glucose values overlap the range of parental control values and all are well within the range of individual animal values for reference controls… Furthermore, the two MON863 group mean values are only slightly higher than the average historical glucose level for this strain of rat that was utilized in other toxicology studies performed by Covance Laboratories [who performed the study].*"

Greenpeace says: Once again, Monsanto is relying on reference ranges and historical means for the rats, rather than focussing in on the critical statistical significant differences between MON863 fed rats and the control. Surely it is important to know with certainty whether the increases on serum glucose levels are "attributable to MON863" or not. What scientific data is present to determine that these are "of no biological consequence", except for the reference values and historical means.

8) Statistically significant difference in kidney weights
High dose (33 % MON863) male kidney weights were significantly different to the control group. The kidney weight were 7.1 % lower for the high dose compared to the control. The difference was also evident when the kidney weights were normalised for either body or brain weight.

Monsanto says: "*Statistical analysis of the male high dose, parental control and reference control kidney weight data in an analysis of variance ANOVA p value …[which] does not meet the predefined criteria for rejection of the null hypothesis of all group means being equal. The differences between male treated and control kidney weights is small (i.e. < 10 %) and all individual MON863-fed values fall within the range of reference controls values. The absence of changes in blood urea nitrogen and creatinine levels in male kidneys are corroborating evidence for the lack of adverse effect on renal function.*"

Greenpeace says: ANOVA should not have been used here. ANOVA is used to determine whether a variable is significantly different between a number (>2) of experimental groups. The "reference" control is not part of the experiment so cannot be included. The reference group has a wider range than the control group. The effect of including this wide ranging group of values is to increase the variability (variance), thus obscuring the significant difference found between the high dose and control group. As Monsanto state, "*the purpose of including these additional reference control groups was to establish a normal range of values for all the parameters measured.*" Thus, it is clear that they should not be included in the statistical comparisons drawn from the study. Once again, the use of reference values is used to mask the significant differences found in the study. In addition, why has Monsanto performed ANOVA only on this variable and not on any of the other variables (although ANOVA would equally invalid for any of the other variables). This is, at best, an inconsistent statistical approach.

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The percentage difference may be smaller than seen in the other significant differences, but it is still significant. If blood urea nitrogen and creatinine levels in male kidneys are unchanged, then data should be presented to demonstrate this to ensure if is a statistically valid statement. Further studies

should be conducted to find the cause of the underweight kidneys and, importantly, to confirm the apparent "lack of adverse effect on renal function" in long term experiments.

9) Different renal tubular mineralization
The high dose (33 % MON863) females rats had significantly lower renal tubular mineralization.

Monsanto says: *"Even if this were regarded as a treatment related effect, it would not be considered adverse. In this study, tissues from the reference control animals were not processed for histopathological examination. However, Monsanto does have historical control pathology data from five additional 90-day maize feeding studies with the same strain of rat…In the majority of circumstances where the incidence of a microscopic finding was higher in the MON863 group than the parental control, the MON863 incidence was actually lower than that observed in one or more control group from previously conducted studies."*

and also, *"The incidence of three microscopic findings in MON863 male rats is higher than observed in both the parental and Monsanto historical group findings…Neither of these differences is statistically significant."*

Greenpeace says: This is not a statistically valid comparison. Firstly, no comparison can be made because the control group is not part of the experiment. Only the vaguest of inferences can therefore be made from the data. Secondly, there does not appear to be any statistical treatment of the data (it is explicit that means are provided; no standard deviations nor ranges nor p values are presented). Therefore, for the female rats the comparison between the previously conducted studies is invalid. For the male rats, it is not clear how the conclusion that these findings are not statistically significance is arrived at.

The reason why the control group from the study was not processed for histopathological examination is not given. However, the data show that there may be some differences in the microscopic findings, but this will only become clear with a proper, statistically valid comparison between the appropriate dose and control groups.

Conclusion

Monsanto has tried to refute these 5 statistically valid differences by using "reference" and "historical" values, which have not formed part of the experimental study. The inclusion of these "reference" and "historical" values is not appropriate as the critical comparison is between the GE maize fed animals and the experimental controls.

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