TESTBIOTECH Background 18 - 11 - 2014

Comments regarding the GRACE open letter to Testbiotech in response to its report and press release dated 7-11-2014[1]

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In a comment on Testbiotech's report of 7 November 2014, Prof. Dr. J. Schiemann, coordinator of the GRACE consortium, repeats what the authors of the GRACE publication Zeljenková et al. (2014) have already stated. However, repeating this statement does not eliminate observable differences between animals treated with MON810 and the control group.

The GRACE consortium asserts that "Testbiotech's comments fail to distinguish between statistical significance and biological relevance". This assertion has no basis at all. In fact, there are numerous statistically significant differences in the GRACE study where Testbiotech agrees that they have no biological relevance, and which therefore have not been mentioned in Testbiotech's report. For the three parameters for which statistically significant **and** biologically relevant effects were identified, the Testbiotech report provides substantial reasons, i.e. dose-dependent changes, a consideration of individual data and references.

1. Detailed replies to the GRACE letter concerning total serum protein (TP)

I. The changes in TP are inconsistent across groups

The statement that the changes in TP are "inconsistent" just repeats what has been stated by Zeljenková et al. (2014) and what is in fact at the core of Testbiotech's critique. This critique focused on the dismissal of a dose-dependent, statistically significant decrease in TP as not being "related to the feeding of the GMO-containing diets" (Zeljenková et al. 2014). In fact, this decrease was observed in both trials. The only difference was that it was observed in males in Trial A whereas it was seen in females in Trial B. Does the fact that this decrease was observed only in one sex, though different each time, invalidate the fact that it was **both dose-dependent and statistically significant** in each experiment? This appears to be a far-fetched claim of "inconsistency". It is a well-known fact that due to the "intangible variance" of biological systems (Gärtner 1990) it is rarely possible to reproduce effects in a congruent manner. Furthermore, clinical pathology was only investigated at the end of the study. Therefore, it is possible that the observed decrease in TP only started when the study ended. This could explain why it was observed only in males of Trial A and only in females of Trial B. It could also explain why this decrease was not reflected by a decrease in body weight, although such a decrease does not necessarily need to happen.

[1] Expertise was made available by a toxicologist with long-term experience in regulatory toxicology.

II. A decrease of TP was also seen in female rats of Trial B fed conventional maize

While it is true that a statistically significantly lower TP level was observed in female rats in the conventional group 2 of Trial B, Prof Schiemann should stay true to his own principles, i.e. discern statistically significant from biologically relevant changes. It is not appropriate to use a single, and even less pronounced difference to dismiss a dose-dependent, statistically significant decrease seen in two independent trials. More importantly, the GRACE consortium remains completely mute about individual animals, which were in the pathological range of TP values, i.e. lower than 50 g/L. A total of five MON810-treated animals have been identified with such low levels, values which were not seen in any animal of any other group in both trials.

III. The magnitude of differences between groups was considered to be small

Whether a certain magnitude of group differences is considered small or not may be a matter of debate. However, the plain statement that a 10-20% difference "was considered to be small" does not add much to the debate. Rather an explanation should have been provided why a 20% decrease should be considered "small", when a 22% decrease was observed as part of the changes in an established disease model (Palanisamy et al., 2008).

IV. One should expect a decrease in body weight together with decreased TP levels

A decrease in body weight may or may not be seen. In fact, Palanisamy et al. (2008) described a significant **increase** in body weight together with a significant decrease in TP. In addition – as mentioned above – an impairment of the synthesis capacity of the liver, which may just have been starting at the end of the study would perhaps not yet be reflected by a decrease in body weight. Furthermore, due to the lack of urine analyses, it is difficult to know whether the decrease in TP was due to an impaired liver function or due to renal toxicity.

V. Testbiotech did not acknowledge that histopathological alterations were not seen in liver and kidney

In fact, Testbiotech took note of the statement by Zeljenková et al. (2014) that no histpathological alterations were observed. That is why Testbiotech stated that "it remains unclear whether generalised oedema were not present, forgotten to report or not noticed". Although it rarely happens, it sometimes occurs that pathological changes go unnoticed. Therefore, it seems reasonable to ask for a re-assessment of the histopathological slides of those 12 animals, which had TP values lower than 20% compared to their respective control group means. It should be noted that the nephrotic syndrome is characterised by generalised oedema. Therefore, Schiemann's restriction of expecting "oedema … of the kidneys" is wrong.

2. Detailed replies to responses of the GRACE Consortium concerning blood glucose and pancreas weight

The response does not contain a reply to Testbiotech's major critique that Zeljenková et al. (2014) "did not discuss these changes (i.e. the increase in blood glucose and the decrease pancreas weight) in conjunction, in spite of the well-known role of the pancreas in the regulation of blood glucose levels." Instead, GRACE repeats what already had been stated in the publication and what was actually criticised by Testbiotech. Testbiotech drew attention to the fact that there is a **dose-dependent** decrease in relative pancreas weight of male rats **in both trials,** which in addition was statistically significant in Trial A. Moreover, the significantly lower pancreas weights in Trial A were accompanied

by significantly higher blood glucose levels strongly suggesting that these changes were not coincidental. Zeljenková et al. (2014) as well as Schiemann avoid a discussion of this observation. Rather Schiemann again refers to data of the conventional maize varieties, emphasising that they too had lower pancreas weights. What he again forgets to point out is that the decreases seen in the conventional maize varieties are clearly less pronounced (i.e. 5% and 14%) than the decreases seen in the MON810-treated animals (i.e. 18% and 21%). It remains to be elucidated what caused the significant decrease in pancreas weight in the animals fed with the conventional 33% SY-NEPAL variety, animals which by the way also had a slightly higher blood glucose level. However, this finding does not invalidate the results described above.

Conclusion

The open letter from GRACE consortium does not contain any arguments or facts which might lead to the abandonment of the conclusion drawn by Testbiotech that treatment with MON810 under the study conditions of Zeljenková et al. (2014) caused biologically relevant, statistically significant, dose-dependent effects on total serum protein and relative pancreas weight, the latter accompanied by an increase in blood glucose levels. Therefore, Testbiotech's general conclusion remains unchanged: Zeljenková et al. (2014) were unable to determine a no-observed effect level (NOEL) for MON810 under the experimental conditions they used.

References

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