G-TWYST
GM PLANTS TWO YEAR SAFETY TESTING

Answer to the Comments by the Federal Office of Consumer Protection and Food Safety (Germany)

28 November 2016

First of all we would like to thank the Federal Office of Consumer Protection and Food Safety (BVL) for the very constructive comments regarding our publication in which we proposed criteria for the evaluation of the scientific quality of mandatory rat and mouse feeding trials with whole food/feed derived from genetically modified plants (Schmidt et al., 2016a). We generally agree with the received comments, but would like to take the opportunity to further discuss particular issues regarding the above-mentioned publication, which have been raised by BVL, as follows:

1) Although only 90-day animal feeding trials but not long-term animal feeding studies are currently a mandatory requirement to assess the risk of genetically modified food/feed according to the Implementing Regulation No. 503/2013/EC, we are of the opinion that the proposed criteria should not only apply to the 90-day feeding trials but also to long-term feeding trials falling under the EFSA scenario 2 intended to support the risk assessment of a particular genetically modified food/feed. By doing so, we think that the quality of long-term studies as well as the acceptability of such studies by the regulatory bodies will be enhanced.

2) BVL states that our publication fails to give examples on what might trigger a scientifically justified necessity for a long-term animal feeding trial with genetically modified food/feed. We have to clarify that the G-TwYST project consortium will discuss the triggers that might justify the performance of a long-term feeding trial with genetically modified food/feed once the results of the 90-day as well as the combined chronic toxicity/carcinogenicity study with the genetically modified maize NK603 are available, i.e. at the end of the project.

3) BVL asks whether it is really necessary to test both the starting material and the diets for the presence of the event at any rate. The objective of this approach is to allow for a

comprehensive documentation of the quality of the test and feed material, which is considered crucial for contentious issues. The analyses shall ensure that test material and diets do not accidentally deviate from the intended composition/test design.

4) BVL disagrees with our proposal that the presence of (all existing) other events as background contamination in the whole food/feed must necessarily be tested. In their opinion, small admixtures of seeds with events other than the introduced event are equated with accidental admixtures from other non-genetically modified varieties and, therefore, do not affect the risk assessment due to their low proportion. The purpose of the analyses is to confirm that test material and diets do not accidentally deviate from the intended composition/test design. It is acknowledged that small admixtures may usually not affect the risk assessment.

5) We proposed that the highest level of the plant material that can be incorporated in the animal diets without leading to a nutritional imbalance is tested. As stated by BVL, our proposal differs from that of EFSA, which recommends two dose levels as a minimum requirement for long-term animal feeding trials with genetically modified food/feed (EFSA, 2013). In practice (i.e. in the case of the animal feeding trials performed in the frame of the GRACE project), two dose levels indeed helped in the interpretation/evaluation of results in order to distinguish between treatment and non-treatment related effects. We agree with BVL that this point needs to be further discussed and animal feeding trials with genetically modified food/feed such as those being currently performed in the frame of the G-TwYST project as well as further animal feeding trials being performed elsewhere might serve as a basis to decide which might be the best choice.

6) A literature search to get adequate incorporation rate data for a certain crop, as pointed out by BVL, might help in deciding which level of the plant material can be incorporated into the animal diets. When wanting to do so, one should take into account whether the study/studies on which one bases its decision has/have been performed according to internationally accepted guidelines and complies/comply with the proposed quality criteria described by Schmidt et al. (2016).

7) An acceptable inclusion rate finding study could be based on a 28-day feeding trial by taking into account the OECD Test Guideline No. 407 (OECD, 2008) and the quality criteria proposed by Schmidt et al. (2016).

8) In the context of the criterion “A non-GM line with a comparable genetic background is used as a control”, BVL wants the consortium to specify what is meant by a “well established history of safe use”, since the near-isogenic control line is not a commercially used line. In our opinion, the near-isogenic line should be a line from an established plant breeding program and, therefore, is closely related to commercially non-GM varieties already cropped.

9) In the context of the criterion “A non-GM line with a comparable genetic background is used as a control”, BVL wants the consortium to comment on the natural variability of non-GM reference lines. The natural variability depends on the kind of crop and can be large, considering e.g. a broad list of sexually propagated/bred varieties and varieties adapted to specific regional conditions. If considered for testing, non-GM references should be representative varieties used for cropping (in the EU or growing/export region).

10) BVL argued that the publication did not include a statement regarding the use of strain-specific data from the scientific literature to get information on the natural variability of the endpoints. In this context, BVL indicated that the consortium should define what is accepted as proper historical background data and where to get this information from. The consortium is of the opinion that information on the variability of the endpoints should primarily be obtained from historical control data from the same testing facility and from animal feeding studies in which the diet composition was similar to the one used in the feeding study being presently performed. Moreover, the consortium agrees with EFSA (2013) that in case that the diet formulation used in the experiment for the control groups cannot be demonstrated to be equivalent to that used for the generation of historical control data, the inclusion of a further control group (as similar as possible to the historical controls), in addition to the concurrent control group(s), should be

considered. In the frame of the G-TwYST project, the data to be obtained in the control groups of the different feeding trials will be compared with those of the control groups obtained in the frame of the GRACE project (Schmidt et al., 2016b) to get further information on the natural variability of the endpoints and how the composition of the control diets might influence the different haematology and clinical biochemistry parameters.

11) BVL agreed that appropriate randomization techniques are very important and commented that recommendations/criteria on how to perform an adequate randomization would be welcomed. The importance of randomization has recently been pointed out by e.g. Hirst et al. (2014). The perhaps most helpful reference on how to perform an adequate randomization in the context of rat and mouse feeding trials with whole food/feed derived from genetically modified plants are the G-TwYST study plans (https://www.g-twyst.eu/reports/g-twyst-study-plans), which include a precise description of the randomization in the corresponding "Experimental Design" sections. Further valuable contributions are the publications by Festing and Altmann (2002), Kang et al. (2008) and Cressey (2016).

12) There is a considerable consensus of opinion among toxicologic pathologists that implementation of a blinded evaluation during the initial evaluation of tissues can have a negative impact on both the time it takes to accomplish the microscopic evaluation as well as the quality of the information obtained from the study (Crissman et al., 2004). There is a concern that a blinded evaluation makes the task of separating treatment-related changes from normal variation more difficult. In addition, there is concern that a blinded review during the initial evaluation may result in missing subtle lesions. It is felt that an awareness of the treatment group assignment, particularly knowledge of which animals have been assigned to the untreated or other control group, allows the pathologist to intensely focus the histopathologic evaluation and to find important, and sometimes subtle, differences between the tissues of treated and untreated animals. Overall, toxicologic pathologists feel that an awareness of treatment group favors the finding of all treatment-related effects and enhances the accuracy of the histopathological evaluation. Because it has the potential to limit the pathologist's

awareness of normal variation and effects of the experimental design, a blinded evaluation has traditionally been reserved for reevaluation of findings in specific tissues.

References

Cressey, D. (2016) Web tool aims to reduce flaws in animal studies. Nature 531: 128.

Crissman, J.W., Goodman, D.G., Hildebrandt, P.K., Maronpot, R.R., Prater, D.A., Riley, J.H., Seaman, W.J., Thake, D.C. (2004) Best practices guideline: toxicologic histopathology. Toxicol. Pathol. 32: 126-131.

EFSA (2013) Considerations on the applicability of OECD TG 453 to whole food/feed testing. EFSA J. 11: 3347.

Festing, M.F.W., Altman, D.G. (2002) Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J. 43: 244-258.

Hirst, J.A., Howick, J., Aronson, J.K., Roberts, N., Perera, R., Koshiaris, C., heneghan, C. (2014) The need for randomization in animal trials: An overview of systematic reviews. PLoS ONE 9: e98856.

Kang, M., Ragan, B. G., Park, J.-H. (2008) Issues in outcomes research: an overview of randomization techniques for clinical trials. J. Athl. Train. 43: 215-221.

OECD (2008) Test No. 407: Repeated dose 28-day oral toxicity study in rodents. DOI: 10.1787/9789264070684-en.

Schmidt, K., Döhring, J., Kohl, C., Pla, M., Kok, E.J., Glandorf, D.C., Custers, R., van der Voet, H., Sharbati, J., Einspanier, R., Zeljenková, D., Tulinská, J., Spök, A., Alison, C., Schrenk, D., Pöting, A., Wilhelm, R., Schiemann, J., Steinberg. P. (2016a) Proposed criteria for the evaluation of the scientific quality of mandatory rat and mouse feeding trials with whole food/feed derived from genetically modified plants. Arch. Toxicol. 90: 2287-2291.

Schmidt, K., Schmidtke, J., Schmidt, P., Kohl, C., Wilhelm, R., Schiemann, J., van der Voet, H., Steinberg, P. (2016) Variability of control data and relevance of observed group differences in five oral toxicity studies with genetically modified maize MON810 in rats. Arch Toxicol., in press.