

**G-TwYST: Combined chronic toxicity/carcinogenicity NK603 Feeding  
Trial Study Plan**

**Supplementary Information**

**Maize and diet production as well as maize and diet analyses**

**Test Site 1:**

Details will be added later by amendment.

**Maize production**

**Test site 2:**

Details will be added later by amendment.

**Diet production**

**Test Site 3:**

Details will be added later by amendment.

**Maize and diet analyses**

DRAFT

1 **Test and control crops**

2 [Both test and control maize varieties were alternatively grown at two different regions in North  
3 America. The test material will be selected after the quality of both harvests has been tested. Crop  
4 management and cropping conditions were recorded. The appropriate information will be  
5 provided/added once the test material has been identified.]

DRAFT

## 6 **Analyses of feed materials**

7 [Analyses of maize material and diets will be performed in certified laboratories. The material will be  
8 tested for nutrient and antinutrient compounds, further contaminations and presence of GMOs.  
9 Currently tenders are pending. The detailed information will be amended after contracting.]

- 10 • Maize: The key parameters for the analysis of maize will include:
  - 11 ○ Chemical composition
    - 12 ▪ macronutrients & fibre (ADF, NDF, dietary)
    - 13 ▪ minerals
    - 14 ▪ vitamins (A, B, C, E), zeaxanthine
    - 15 ▪ amino acid composition (including tryptophan)
    - 16 ▪ fatty acid composition
    - 17 ▪ antinutrients (phytic acid, trypsin inhibitor)
    - 18 ▪ other secondary metabolites (e.g. phenolics and sterols) and carbohydrates (e.g.  
19 raffinose and stachyose)
  - 20 ○ GMOs (DNA)
  - 21 ○ CP4 protein
  - 22 ○ Chemical contaminants
    - 23 ▪ pesticide residues
    - 24 ▪ mycotoxins
    - 25 ▪ heavy metals and
    - 26 ▪ other contaminants (e.g. dioxins, PAHs, PCBs, nitrate and nitrosamines)
- 27 • Diets: The analysis of diets will be performed on pre-irradiation samples, while post-irradiation  
28 samples of the diets will be stored as back-up for possible follow-up analyses if required. The  
29 analysis of the diets will be performed as follows:
  - 30 ○ The first batch of each dosage group will be checked for the same parameters as for maize  
31 plus the following analytes linked to the presence of soy:
    - 32 ▪ isoflavones
    - 33 ▪ lectins
  - 34 ○ Follow-up batches [analysis strategy depends on number of batches and costs].

35 The maize and the diets will also be subjected to a microbiological analysis. Both maize and diets  
36 (pre- and post-irradiation) will be analysed for the presence of microorganisms, in particular bacteria  
37 (including coliforms, enterobacteriaceae, and specific bacteria), in all samples as well as the presence  
38 of other microorganisms (including yeasts and moulds) in the diets.

39 Regarding the outcome of the analytical studies, an internal report will be prepared, compiling and  
40 summarizing the results of the analyses of maize and diets from laboratories employed.

## 41 **Supplementary analyses of diets at other Test Sites**

42 Omics analyses of plant tissues will be performed by CRAG/UdG.

43 **Diet formulation, sampling and shipping**

44 [Tenders for diet preparation/purchase are currently prepared/pending. Detailed information about  
45 storage, handling and transport will be amended after contracting.]

46 Storage of the test diet during the study: in a closed storage room (cool and dry, controlled  
47 temperature and humidity) of the Laboratory of Toxicology, Slovak Medical University, Limbová 14,  
48 Bratislava, Slovak Republic. The temperature and humidity of the room will be recorded and the  
49 records will be kept. The test diets will be provided as single batches (containing portions of diets  
50 packed in separate vacuum-treated, gamma-irradiated packs).

DRAFT