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: Maize production
Details will be added later by amendment.

Test site 2: Diet production
Details will be added later by amendment.

Test Site 3: Maize and diet analyses
Details will be added later by amendment.
Test and control crops

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

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

- Maize: The key parameters for the analysis of maize will include:
  - Chemical composition
    - macronutrients & fibre (ADF, NDF, dietary)
    - minerals
    - vitamins (A, B, C, E), zeaxanthine
    - amino acid composition (including tryptophan)
    - fatty acid composition
    - antinutrients (phytic acid, trypsin inhibitor)
    - other secondary metabolites (e.g. phenolics and sterols) and carbohydrates (e.g. raffinose and stachyose)
  - GMOs (DNA)
  - CP4 protein
  - Chemical contaminants
    - pesticide residues
    - mycotoxins
    - heavy metals and
    - other contaminants (e.g. dioxins, PAHs, PCBs, nitrate and nitrosamines)

- Diets: The analysis of diets will be performed on pre-irradiation samples, while post-irradiation samples of the diets will be stored as back-up for possible follow-up analyses if required. The analysis of the diets will be performed as follows:
  - The first batch of each dosage group will be checked for the same parameters as for maize plus the following analytes linked to the presence of soy:
    - isoflavones
    - lectins
  - Follow-up batches [analysis strategy depends on number of batches and costs].

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

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

Supplementary analyses of diets at other Test Sites

Omics analyses of plant tissues will be performed by CRAG/UdG.
Diet formulation, sampling and shipping

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

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