G-TWYST

GM PLANTS TWO YEAR SAFETY TESTING

INTERIM REPORT

15 DECEMBER 2016

SEVENTH FRAMEWORK PROGRAMME

Acknowledgment and Disclaimer

The authors of this document thank all project partners for their valuable contributions and comments on draft versions of this document.

This document expresses the view of the G-TwYST consortium, and does not reflect an official opinion of the European Commission. Responsibility for the information and views expressed therein lies entirely with the authors.

In the whole document, the acronym "**G-TwYST**" has been used to refer to the project.

"GMP Two Year Safety Testing" (G-TwYST) is a Collaborative Project of the Seventh Framework Programme of the European Community for Research, Technological Development and Demonstration Activities.

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FEED PRODUCTION AND PLANT ANALYSIS

OBJECTIVES AND TASKS

OBJECTIVES:

- Ensure the supply of plant and feed material throughout the project
- Provide control of the plant and feed quality
- Comparison of the omics characteristics of the crop plant material with those of other GM and conventional maize crops

TASKS:

1. Logistics for feed materials (Lead: JKI)
2. Analyses of plant material (Lead: CRAG)
3. Data collation and bioinformatic analysis (Lead: CRAG)

LOGISTICS FOR FEED MATERIALS

Storage of maize kernels and feed production

Due to the cooperation with the French project GMO90+, which is coordinated by TOXALIM - INRA (Toulouse, France), GM maize NK603 with and without Roundup treatment and its near-isogenic conventional counterpart were cropped and bought from growers in the USA and in Canada, who assured a traceable quality, so that alternative maize material was available. In November and December 2014 the harvested and dried maize kernels were shipped to a storage facility in Germany that offered HACCP compliance. Based on a first set of quality analyses, the Canadian harvests were chosen for the further feeding trials performed throughout the G-TwYST project due to the fact that they showed the lowest levels of mycotoxins, pesticides and other toxic compounds. Low levels of a few transgenic traits (other than NK603) typical for Canada were detected in each commodity (random detection in samples). NK603 is estimated to be present in the near isogenic control variety at ~1%, as estimated by DNA detection and protein detection (comparison of a series of dilutions obtained from NK603 and “non-transgenic” kernel samples through qualitative immunological assays).

After a public call for tender and contracting of the diet supplier, 1 ton of each test material was shipped for processing. The first batch of the test diet was received in July 2015. Further batches were/will be produced on demand (depending on their consumption in the trial) and hence delivery dates were not fixed a priori.

In August 2015 an infestation of the stored maize material with a pest moth (*Plodia interpunctella*) was monitored. Surplus maize from USA was burned. The Canadian maize kernels were fumigated with PH_4, a pesticide that is allowed for application in food storages and does not interfere further on with the feeding trials. Maize samples have been analyzed regarding their general quality and potential contamination with diverse toxicants after fumigation for their further use in the trials. The big bags of maize were transferred to another storage facility and further monitored by JKI personnel. In April 2016 mice were attacking the big bags. The kernels have been cleaned (sieving) and repacked into rigid containers ready for shipment. Surplus maize was burned.

The analysis strategy for the diets has been modified. The strategy that will be followed further on will ensure financial flexibility as well as sufficient quality control for the maize and the diets. By using the lab facilities at the Wageningen Research Foundation (WR) to perform part of the analyses instead of subcontracting a reduction of costs is achieved. The maize material was analyzed before the start of the trials to decide which harvest to use and after fumigation with PH_4 to control the quality of the material.

The first batch of diets was analyzed by a subcontracted certified lab without any critical findings. The part of the analyses performed by WR is pending. The producer of the diets provided/will provide analyses of the "basal mix" i.e. the mixed ingredients of the diet other than the test material. The basal mix is used for the production of two batches of diets. Therefore, combining the analyses from the maize and the basal mix will allow assessing the quality of the diets throughout the project. Up to now, four batches were delivered to the test facility. Samples from each batch of diet have been stored at -80°C for later analyses.

Analyses of plant material

The objective was to compare the omics characteristics of the NK603 plant material with that of conventional maize crops. The two sets of genetically modified maize samples with and without Roundup treatment and its near-isogenic conventional counterpart, cropped in the USA and in Canada (see above), were analyzed. The genetically modified varieties were Prairie Brand and Pioneer 8906 882RR2, respectively.

Proteomics analyses were carried out by resolving whole grain protein extracts in a two-dimension isoelectrofocusing and SDS polyacrylamide gel electrophoresis (2D SDS PAGE) analysis, with pI and molecular weight ranges of 4-7 and 14-250 kDa, respectively, and by silver staining. For each sample, two biological and two experimental replicates were analyzed.

Transcriptomics analyses were performed with high-quality maize embryo RNA (RNA integrity number [RIN] above 8 and rRNA ratio above 2). Next Generation Sequencing RNA-Seq was carried out at CRAG (Barcelona Scientific Park) with the Illumina approach. Fifty basepair paired ended reads were generated with a 40 M reads/run depth.

DATA COLLATION AND BIOINFORMATIC ANALYSIS

Proteomics

The Melanie classic/DIGE 8 software (Swiss Institute of Bioinformatics) was used for data analysis. After quality control of all gel images, they were aligned to remove the positional variation inherent to the electrophoresis process. Automatic alignments were manually reviewed. Spots were then detected and their intensity was quantified in each gel. A total of 1,200 spots were automatically identified. After manual editing process and ratiometric normalization, 1,045 spots were used for comparison of the different samples.

In the Principal Component Analysis (PCA) of the spot intensities in the various samples only Principal Components PC1 and PC2 had Eigenvalues above 10%. PC1 explained 21% of the overall variability and clearly separated the proteomes of the samples corresponding to Pioneer 8906 (treated and untreated with Roundup Ready, and near-isogenic), cropped in Canada, and those corresponding to

Prairie Brand 882RR2 (treated and untreated with Roundup Ready, and near-isogenic), cropped in USA. Thus, the two groups of samples had different genetic backgrounds (varieties) and grew under different environments. Similarly, a cluster dendogram showed two clear clusters equivalent to the mentioned PC1 groups. Within each cluster, genetically modified or conventional varieties did not form specific sub-clusters, indicating no substantial changes in the genetically modified kernel proteome.

Transcriptomics

Quality control for raw Illumina reads was performed with the standard tool FASTQC software, and no significant issues were detected. Adaptor and vector sequences, poly A tails as well as contaminant rRNA (around 10% reads) were trimmed. Cleaned reads were mapped to the *Zea mays* reference genome assembly using HISAT2. Having obtained an alignment file, the gene annotation of the reference genome was used to calculate the number of reads mapping each gene in the different analyzed samples. We performed alignment and count data quality control and applied GC bias correction. The GC-normalized count data set was subjected to the standard TMM normalization method to correct undesired effects due to the different sample sizes of the samples.

Overall, 84% maize genes were mapped by at least 1 count per million reads (CPM) in at least one sample, which represents the sensitivity of the approach. At the same time, only about 40% maize genes were mapped by at least 5 CPM in at least one sample. Grains from mature and dry embryos seem to present this type of transcriptomic profile. In order to compare the transcriptomes of the different samples, we filtered low-count genes, as it is required in differential expression quantification packages such as Limma.

As one would expect, the cp4epsps transgene was highly expressed in NK603 samples (it was among the 25% of genes with the highest expression).

No differentially expressed genes (DEG) were found when comparing the genetically modified and near-isogenic varieties. However, a specific comparison of genetically modified and near-isogenic samples of the same variety and grown at the same location showed 10 and 8 DEGs for Prairie Brand (Canada) and Pioneer (USA), respectively. This corresponds to 0.02% of the maize genes and did not preferentially belong to any given biological process. There were no commonly regulated genes.

On the one hand, a comparison of herbicide treated and untreated crops did not reveal any DEG. On the other hand, the environment and/or the natural variability accounted for 3614 DEG (i.e. around 10% of the maize genes) that were mainly related to stimuli (temperature, water, etc.).

SIGNIFICANT RESULTS

Test material with the necessary quality is available. Transcriptomics and proteomics data indicate a strong effect of the environment and/or the natural variability of maize material, with residual effects of the herbicide treatment and the genetic modification.

FEEDING TRIALS

OBJECTIVES AND TASKS

OBJECTIVES:

- Perform a 90-day feeding trial with GM maize NK603 (maximum incorporation rate: 33%) according to the OECD Test Guideline 408 and the EFSA Guidance on conducting a 90-day oral toxicity study in rodents on whole food/feed.
- Perform a 90-day feeding trial with GM maize NK603 (maximum incorporation rate: 50%) according to the OECD Test Guideline 408 and the EFSA Guidance on conducting a 90-day oral toxicity study in rodents on whole food/feed.
- Perform a combined chronic toxicity/carcinogenicity study with GM maize NK603 (maximum incorporation rate: 33%) according to the OECD Test Guideline 453 and the EFSA considerations regarding the applicability of OECD TG 453 to whole food/feed testing.

TASKS:

1. 90-day feeding trial with GM maize NK603 at a maximum incorporation rate of 33% (Lead: SZU)
2. 90-day feeding trial with GM maize NK603 at a maximum incorporation rate of 50% (Lead: SZU)
3. Combined chronic toxicity/carcinogenicity study with GM maize NK603 at a maximum incorporation rate of 33% (Lead: SZU)

90-DAY FEEDING TRIAL WITH MAIZE NK603 AT AN INCLUSION RATE OF 11 AND 33%

In the frame of WP3, the first 90-day feeding trial with the genetically modified maize NK603 at an inclusion rate of 11 and 33% ended June 2016. In addition to the "classical" toxicologically relevant parameters described in the OECD Test Guideline 408, immunological and endocrinological examinations were performed in accordance with the study plan. The daily activities involved periodical health status observations, handling of moribund animals and detailed physical examination and functional assessment of the rats, weighing of the animals and food, the changes of drinking water and the bedding material, the handling of the metabolic cages, the cleaning, sanitation, sterilization and extensive filling of the forms as well as all daily records of the primary documentation required when working under GLP conditions. At the end of the study, samples were collected for the following analyses: haematology, clinical chemistry, immunology, endocrinology, urinalysis and histopathology. Specimens taken from the animals included blood, urine, vaginal smears and tissues/organs. Blood samples were divided for the haematology, clinical chemistry and immunology analyses. Tissues/organs were removed and sent to the Department of Pathology, University of Veterinary Medicine Hannover, Germany, where the tissue samples were embedded in paraffin, cut and stained. Thereafter, the stained tissue slices were forwarded to the Laboratory of Histopathology at Roger Alison Ltd. The database with all obtained results has been prepared and at the present time the concordance of the data with the primary documentation is being double-checked.

CHRONIC TOXICITY STUDY WITH GM MAIZE NK603

In September 2016 (i.e. after 12 months), the chronic toxicity part of the combined chronic toxicity/carcinogenicity feeding study with NK603 maize was finalized at the SPF animal facility of the

Slovak Medical University. Chronic toxicity feeding study with NK603 maize was conducted in compliance with the study plan. The daily activities involved periodical health status observations, handling of moribund animals and detailed physical examination and functional assessment of the rats, weighing of the animals and food, the changes of drinking water and the bedding material, the handling of the metabolic cages, the cleaning, sanitation, sterilization and extensive filling of the forms as well as all daily records of the primary documentation required when working under GLP conditions. Tissues/organs were removed and sent to the Department of Pathology, University of Veterinary Medicine Hannover, Germany, where the tissue samples were embedded in paraffin, cut and stained. Thereafter, the stained tissue slices were forwarded to the Laboratory of Histopathology at Roger Alison Ltd. At the present time, the database with all obtained results is being prepared.

QUALITY ASSURANCE AND GLP COMPLIANCE

No deviations from the corresponding study plan occurred during the performance of both feeding trials. Studies were regularly inspected by the Head of the Quality Assurance Unit at the Slovak Medical University.

Studies were conducted in the animal housing facility of the Slovak Medical University. The Laboratory of Toxicology of the Slovak Medical University (certificate No. G-036) received a statement of GLP compliance from the Slovak National Accreditation Service. The Laboratory of Clinical Chemistry of the Slovak Medical University received an accreditation certificate (M-013) from the Slovak National Accreditation Service. The laboratory takes part in the National Quality Control Programme for Clinical Biochemistry. Moreover, the Quality Assurance Unit (QAU) of the Slovak Medical University inspects it. All procedures performed by the Laboratory of Toxicology are described in standard operating procedures (SOP) approved by the QAU. During the present reporting period, the test facility underwent a GLP inspection by inspectors of the Slovak National Accreditation Service.

The studies were conducted in accordance with the OECD Principles of Good Laboratory Practice, as revised in 1997 (ENV/MC/CHEM [98]17), and the EU Commission Directive 2004/10/EC dated 11th of February 2004 (Official Journal No L 50/44). The National GLP Compliance Programme in the Slovak Republic is based on Act No. 67/2010 Coll. and in compliance with the Government Decree No 320/2010 Coll. The studies were also conducted in accordance with EU Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes.

In addition to the performance of the feeding studies, WP3 activities included the collaboration with other project partners and WPs. We attended teleconferences, meetings and common discussions focused on the future strategy of the project.

SIGNIFICANT RESULTS

The first data of the 90-day feeding trial with the genetically modified maize NK603 at an inclusion rate of 11 and 33% and the first data of the chronic toxicity part of the combined chronic toxicity/carcinogenicity study with the genetically modified maize NK603 maize were presented at the GA/PEC meeting held in Wageningen, The Netherlands, on the 7th and 8th of November 2016.

BIOSTATISTICS

OBJECTIVES AND TASKS

OBJECTIVES:

- Perform prospective power analyses to ensure that the animal feeding trials will be fit for purpose.
- Perform statistical analyses of the results from the studies in WP3.]
- Report on the results of the statistical analyses and provide recommendations on the statistical methodology for long-term feeding trials.]

TASKS:

1. Prospective power analysis of the experiments (Lead: DLO)
2. Statistical analysis
3. Recommendations on statistical methodology (Lead: DLO)

PROSPECTIVE POWER ANALYSIS

The originally planned prospective analysis to evaluate the power of difference and equivalence tests for the planned designs and analyses (Task 1) was delayed and modified in line with the overall delay in starting the experiments. The logistics of the experimental design did not allow to set sample sizes based on a prior power analysis. Therefore, the sample sizes were chosen according to OECD Guidelines, and the power analysis will be used to evaluate the effect magnitudes that can be detected with a pre-set power level.

RECOMMENDATIONS ON STATISTICAL METHODOLOGY

For a power analysis, the methodology (testing methods, statistical models, contrast of interest) should be determined first; therefore, the development of an appropriate statistical methodology (Task 3) was given high priority. The Statistical Analysis Plan (SAP) has been developed based on equivalence testing with respect to variation, as observed in previous studies for non-GM varieties. In this methodology, the power of the equivalence tests in case of a zero true effect will at least equal to a pre-set level if the replication and the precision in the current experiment are equal or better than in the historical experiments or as required by regulators.

SIGNIFICANT RESULTS

A methodology for equivalence testing based on external reference data was further developed and tested on data from the GRACE project as available on the CADIMA website. The group coauthored a comparative paper on the GRACE data (open access; see Schmidt, K. et al. (2016) Arch. Toxicol., doi:10.1007/s00204-016-1857-x).

SCIENTIFIC VALUE AND SOCIAL ISSUES

OBJECTIVES AND TASKS

OBJECTIVES:

- Development of criteria to evaluate the scientific quality of long-term feeding studies for GM food/feed risk assessment according to internationally accepted guidelines.
- Identification of possible triggers that would justify a 2-year feeding trial.
- Determination of the added value of long-term feeding trials for GM food/feed risk assessment.
- Identification and characterization of the normative dimensions of the controversy on whole-food animal toxicity studies in GM food/feed risk assessment and investigation how they relate to the scientific dimensions.

TASKS:

1. Evaluation of the scientific quality of long-term GM food/feed feeding trials (Lead: TiHo)
2. Justification of long-term feeding trials for GM food/feed risk assessment (Lead: TiHo)
3. Analysis of the normative dimensions and how they relate to the scientific aspects (Lead: UNI-KLU)

EVALUATION OF THE SCIENTIFIC QUALITY OF LONG-TERM GM FOOD/FEED FEEDING TRIALS

In the first reporting period and as a first step, the members of an expert group identified and discussed 31 different aspects to be taken into account when evaluating the scientific quality of publications on feeding trials with genetically modified plants as feed constituents in rats. In the present reporting period and based on the received comments, a set of nine quality criteria as well as particular issues referring to the individual quality criteria were put together. It is important to note that the described set of key quality criteria applies to 90-day feeding trials as well as to long-term feeding trials such as those performed to test the potential chronic toxicity and/or carcinogenicity of whole food/feed with a duration of 12 to 24 months.

Proposed criteria:

1. *The design of the feeding trial is based on internationally recognized test guidelines, but adapted for specific needs of whole food/feed studies and non-targeted testing.*
2. *An analysis of the plant materials and diets including, among others, macro- and micronutrients, biological and chemical contaminants as well as the identification and quantification of the event, is performed.*
3. *The highest level of the plant material that can be incorporated in the animal diets without leading to a nutritional imbalance is tested.*
4. *A non-GM line with a comparable genetic background is used as a control.*
5. *Specific aspects regarding the choice and housing of the laboratory animals used in the feeding trials are considered.*
6. *Appropriate randomization techniques are applied.*
7. *A reliable and appropriate sample collection and processing strategy is implemented.*
8. *The staff performing the feeding trial and the analysis of the plant materials, diets and animal samples is "blind" with respect to the identity of the diets.*

9. *Appropriate statistical methods are applied to evaluate the power of the study and to analyze the obtained results.*

In the present reporting period a manuscript describing the above-mentioned criteria and particular aspects related to each of them has been published (Archives of Toxicology 90: 2287-2291, 2016).

Moreover, a workshop to discuss the proposed criteria with project team members and external experts from research organizations, competent authorities, and industry took place on the 21st of June 2016 in Quedlinburg, Germany. The invited external participants were Roland Buesen (BASF, Germany), Harry Kuiper (Wageningen University, The Netherlands), Anna Lanzoni (EFSA, Italy), Kevin Leiner (Syngenta, USA), Nathalie Priymenko (Toxalim, ENVT, INRA, Toulouse, France) and Andrea Scheepers (Federal Office of Consumer Protection and Food Safety - BVL, Germany). The workshop included three sessions with the following topics: 1) Presentation and discussion of the proposed criteria; 2) Concepts in statistical methods applied to analyze feeding trials with whole food/feed derived from genetically modified plants as an example; 3) When does a statistically significant difference between two experimental groups become toxicologically relevant? After the meeting, the German Federal Office of Consumer Protection and Food Safety (BVL) forwarded a comment on the proposed criteria. This comment as well as the answer of the G-TwYST expert group have recently been published at the project website.

JUSTIFICATION OF LONG-TERM FEEDING TRIALS FOR GM FOOD/FEED RISK ASSESSMENT

To define the triggers that would justify performing a chronic toxicity/carcinogenicity feeding trial, the WP5 partners will collect scientific literature and guidance documents on 90-day feeding trials from a broad range of toxicity assessment contexts. Previously applied or suggested trigger items will be subjected to a review by the project team and candidate trigger items will be identified. The usefulness of the candidate triggers will be evaluated by analyzing the data obtained in the G-TwYST project with the genetically modified maize NK603 in the two 90-day feeding trials and in the combined chronic toxicity/carcinogenicity study as well as the results of feeding trials having been performed by other research groups/consortia in the past. Recommendations on the application of candidate trigger items will be compiled in a second workshop with external experts and a corresponding report will be published at the project website.

ANALYSIS OF THE NORMATIVE DIMENSIONS AND HOW THEY RELATE TO THE SCIENTIFIC ASPECTS

The overall aim of this task is to gain a better understanding of the issues and the dynamics of the disputes on animal feeding studies and of the possible interrelation among results from scientific experiments and values, world views, or interests.

The overall approach for this task is to compare the controversy on animal toxicity studies in GMO risk assessment to other science-policy conflicts related to animal toxicity studies in food safety, which will be elaborated as case studies. The analytical framework for conducting this analysis and the interpretation of results draws mainly on the literature from science, technology and society studies.

In this reporting period, the following activities were pursued:

- Literature and document review (continued from the first reporting period)

– Elaboration of the draft research plan

Based on a literature and documents review, a draft research outline comprising a total of eight candidate research hypotheses was developed and five candidate case studies were identified and characterized. This document was circulated to expert advisors in preparation of the workshop.

Eight expert advisors were identified based on a literature and document review as well as on experience in the field. Four of them did not participate for different reasons. Four experts from public research institutions agreed to participate as advisors: two social scientists with experience in toxicity assessment, one researcher with a background in technology assessment, and one toxicologist.

All expert advisors met with the project team on the 7th and 8th of October 2016 in Rotterdam to discuss and refine the research outline - in particular, the draft hypotheses to be investigated, the possible case studies/cases to draw on, and the overall approach, methodology, scope as well as the time schedule.

The draft research outline is presently being refined and will be the basis for the empirical step starting in January 2017.

SIGNIFICANT RESULTS

A set of criteria to evaluate the scientific quality of publications on feeding trials with genetically modified plants as feed constituents in rats and mice is available and has been published (open access; Archives of Toxicology 90: 2287-2291, 2016).

CENTRAL ACCESS DATABASE

OBJECTIVES AND TASKS

OBJECTIVES:

- Central storage of project-generated data
- Interlinking research activities and data from different studies
- Promoting open access to project data

TASKS:

1. Integration of G-TwYST into the existing database CADIMA (Lead: JKI)
2. Optimisation of the G-TwYST specific operation of the joint central access database (Lead: JKI)
3. Database maintenance (Lead: JKI)

INTEGRATION OF G-TWYST INTO THE EXISTING DATABASE CADIMA

The web portal CADIMA (Central Access Database for Impact Assessment of Crop Genetic Improvement Technologies; www.cadima.info) was established by the GRACE project. Since then it is operated and hosted by JKI. The portal currently presents the services and outcomes of the EU-funded projects GRACE (public, open access) and PreSto GMO EraNet (public and internal data) to be used for follow up activities (D 6.2).

Regarding the feeding studies with whole GM food/feed, the sets of raw data generated in the course of GRACE are available in the respective service area of the portal (90-day and 1-year feeding trials with MON810 maize). These data are stored in a consistent file format and coding agreed by the projects GRACE (EU FP7, ended in 2015), G-TwYST and GMO90+ (French consortium that will end in 2017) to support their combined use/analyses.

The layout of CADIMA has undergone a few changes to address suggestions from users of the different service areas.

JKI is also hosting the project internal web drive, which allows the safe exchange of internal project documents between the projects.

SIGNIFICANT RESULTS

Databases are online and operating.

COMMUNICATION AND DISSEMINATION

OBJECTIVES AND TASKS

OBJECTIVES:

- Ensure good governance by involving a broad range of stakeholders (industry, professional organisations, civil society organisation, competent authorities, and members of the academia) in key steps of the Project to inform and fine-tune the research process and to enhance relevance of the project results from a broader societal perspective.
- Ensure professional communication on and utmost transparency of what is being done, why it is being done, how it is done, and who is doing it.

TASKS:

1. Stakeholder engagement and communication strategy (Lead: UNI-KLU)
2. Stakeholder consultations*
3. Editorial board (Lead: UNI-KLU)*
4. Project website and electronic networking (Lead: LIS)*
5. Open access journal manuscripts and discussion forum (Lead: TiHo)*
6. Media and policy briefs (Lead: LIS)
7. Presentation of the final report (Lead: LIS)
8. Feed-back on the process (Lead: UNI-KLU)*

Tasks relevant for this reporting period are marked with an asterisk.

STAKEHOLDER CONSULTATIONS, PART I

The main aims of stakeholder engagement are to (i) better inform about the G-TwYST research, its procedures, standards, limitations and context, (ii) inform and guide the research process and the interpretation of results, (iii) promote trust-building among actors.

The main aim of this consultation was to discuss and improve the plans for preparation, conduct and analysis of the animal feeding trials.

The first stakeholder consultation was held as a two-day workshop in Vienna, Austria, on the 16th and 17th of December 2014 and was attended by 41 stakeholders from 14 Member States, USA and Norway and 15 members of the project. The workshop was followed by a written consultation period, which resulted in 131 comments and questions from stakeholders.

These comments and questions were subsequently clustered according to categories and allocated to partners for developing responses. Given that the consultation was held at a later point in time than planned in the Description of Work and that the subsequent reporting and response management was a more complex task than expected, the reporting deliverable was delayed. Moreover, some comments suggesting changes were adopted, resulting in changes in the project plan, and other comments required additional information from subcontractors. These changes required internal clarifications and coordination efforts.

These changes, nevertheless, prove that the consultation process is working very well, resulting in scientifically valid modifications, which are more in line with stakeholder needs.
The final version of the list of comments and responses was published at the project website in June 2016.

STAKEHOLDER CONSULTATIONS, PART II

At the General Assembly 2016 held in Wageningen, The Netherlands, in October 2016, it was decided to split the stakeholder consultation on the draft project results into two subsequent steps:

- One written consultation will focus on the draft results of the two 90-day animal feeding trials, as these two studies are of limited novelty (new issues: event NK603 included, one group with 50% inclusion rate of maize in the diet). This step will be conducted in month 46.
- The consultation on the results of the two-year chronic toxicity/carcinogenicity study and all other draft results in the project will be held as planned at the end of the project in month 47/48.

EDITORIAL BOARD

The Editorial board (composed of the coordinator, the project manager, and the Work package 7 team) reviewed and commented on all relevant project information provided via internet or in publications in the reporting period.

PROJECT WEBSITE AND ELECTRONIC NETWORKING

Several documents and news items were added to the website. These include:

- The draft report of the first G-TwYST stakeholder workshop;
- G-TwYST responses to written comments;
- the final G-TwYST study plans;
- the proposed scientific quality criteria;
- links to GRACE conclusions and recommendations and other GRACE publications.

G-TwYST was extensively highlighted in the media twice:

- On the 24th of July 2015 Hanno Charisius, independent journalist, wrote in “Krautreporter”, a journal for investigative journalism, on the first G-TwYST stakeholder workshop that was held shortly before Christmas in Vienna. Charisius interviewed a number of attendees in the workshop. The article is in German and is available at the “Krautreporter” website.
- On the 12th of October 2016, ARTE broadcasted "OGM - Mensonges et vérités" (GMOs - Lies and truths) and "Vorsicht Gentechnik?" (Take care, Genetic Engineering?) on French and German Television. In this 100 minutes long documentary, Frédéric Castaignède sheds light on genetically modified crops, the impact on farmers, biodiversity and human health. The goals, design and results of GRACE and G-TwYST are pictured between 1:02:00 - 1:18:15. The documentary provides only a ‘snapshot’ of the GRACE Final Conference, which took place on the 9th and 10th of November 2015 in Potsdam, Germany. We refer to videos on the GRACE Youtube channel for a full account. The documentary was discussed in both the “Frankfurter Rundschau” and “Le Monde”.

The G-TwYST Linked-In Group (www.linkedin.com/groups?home=&gid=8186734), created in 2014, provides news and opportunities to discuss events and publications related to G-TwYST. On the 20th of October 2016 the G-TwYST LinkedIn Group had 88 members, representing science, industry, CSOs, Competent Authorities and a few journalists. During the reporting period, 20 new items were added, bringing the total to 44 items.

OPEN ACCESS JOURNAL MANUSCRIPTS AND DISCUSSION FORUM

One journal manuscript on "Proposed criteria for the evaluation of the scientific quality of rat and mouse feeding trials with whole food/feed derived from genetically modified plants" (relevant to Task 5.1) was accepted for open access publication in *Archives of Toxicology* (Schmidt et al., 2016a).

Two manuscripts on the statistical analysis of data from animal feeding studies (relevant to WP4 tasks) were accepted for open access publication in *Archives of Toxicology* (Schmidt et al., 2016b,c).

On top of the discussion forum provided by the journal, an opportunity to publish comments and responses on the project website was established. The main reason was that comments provided to the journal forum require commenters to pay substantial open access fees.

In the course of the reporting period, one comment on the final conclusions and recommendations of the preceding project GRACE (Wögerbauer et al., 2016) was received and answered by the G-TwYST project team (Wilhelm et al., 2016). Another comment was received from the German Federal Office of Consumer Protection and Food Safety (BVL) on the criteria for the evaluation of the scientific quality of rat and mouse feeding trials with whole food/feed derived from genetically modified plants proposed by the G-TwYST consortium. This comment as well as the answer of the G-TwYST expert group have recently been published at the project website.

FEEDBACK ON THE PROCESS

The aim of this task is to learn about the views of the participants (stakeholders as well as project team members) on the participation process and related issues. Results of the first feedback round on planning stage consultation should also be used for fine-tuning of participation in the process.

After completing and publishing the responses to stakeholder comments on the project website, the first feedback round was conducted between project months 25 and 27.

As a first step a conceptual framework for the feedback task, which served as a basis to devise questions, was developed. In a next step, an electronic questionnaire using LimeSurvey was developed. The questionnaire comprised a total of 21 statements on upstream engagement, transparency, accessibility, responsiveness, and reflexivity and asked participants for a 5-scaled rating ranging between "completely agree" to "completely disagree". This questionnaire was sent to all 59 participants of the first stakeholder consultation and yielded 24 responses. The analysis of the responses was discussed with the project and Advisory Board members at the 2016 GA held in Wageningen, The Netherlands, in October 2016. The results were summarized in an internal document, which will become part of D7.7 Feedback report on the process - planned for month 48.

A second round of feedback is planned after/during the stakeholder consultation on the draft results (March-April 2018 - month 47/48). At this stage, interviews with participants will also be conducted.

As an additional feedback instrument, an electronic discussion group was established using the free forum housing platform ZetaBoards, with provocative and partly more general questions on engagement, transparency, communication, responsiveness, and reflexivity - partly in response to stakeholder suggestions. Until the end of the reporting period, no responses were received. The research team will consider whether or not to use this instrument again in the interim consultation on the draft results of the 90-day feeding trials.

SIGNIFICANT RESULTS

The most significant results are the completed Stakeholder Consultation Reports (https://www.g-twyst.eu/reports/workshop-documentys-planning-phase) and the feedback received in Task 8.

Results from the stakeholder consultation

The stakeholder consultation helped in deciding which feeding trials should be performed, thereby leading to the following major changes:

- to perform the original 90-day feeding trial with GM maize NK603 at an inclusion rate of 11 and 33%;
- not to perform the 2-year feeding trial with the GM maize MON810*;
- to perform a further 90-day feeding trial with GM maize NK603 at an inclusion rate of 50%;
- to perform the original combined chronic toxicity/carcinogenicity feeding trial with GM maize NK603 at an inclusion rate of 11 and 33%;
- to analyze potential immunotoxic and endocrine effects in the 90-day feeding trial with GM maize NK603 at an inclusion rate of 11 and 33%.

The main reasons for not adopting suggestions were conflicts with time, budgetary and contractual obligations.

Results from the participant feedback

Participants perceived that the stakeholder consultation process on planning issues enhanced the scientific quality and the clarity of the project plans, brought new insights into others perspectives, increased acceptability and the trustworthiness of the researchers. Positive responses on these issues were very strong with up to 94% of stakeholder participants (100% of project participants). Most dimensions were in the range of 80 to 90% of stakeholder participants strongly or somewhat agreeing, which indicates a high level of satisfaction with the consultation process.

Improvements suggested include employing a less technical jargon, better information on the progress of the project, earlier inclusion of stakeholders (even at call for proposals stage), and allowing stakeholders a role in decision-making. The suggestion on reducing technical jargon will be considered when planning the stakeholder consultations on the draft project results. Other suggestions are either not directly relevant for consideration in the project or are conflicting with legal contract requirements for project funding.