Conclusions and recommendations

on animal feeding trials and alternative approaches and on the use of systematic reviews and evidence maps for GMO impact assessment

GRACE has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 311957.

Project duration: 1st June 2012 – 30th November 2015
GRACE website: www.grace-fp7.eu

Content

Preface and disclaimer

This document compiles the conclusions and recommendations developed in the context of the EU-funded research project GRACE (GMO Risk Assessment and Communication of Evidence) and does not represent an official opinion of the European Commission.

It is subdivided into two major parts:

- Part I covers conclusions and recommendations on animal feeding studies and alternative approaches with regard to Article 12 of the Implementing Regulation (EU) No. 503/2013.
- Part II lays down conclusions and recommendations on the use of systematic reviews and evidence maps when summarizing and evaluating GMO impact data.

No liability can be accepted by the authors for material or immaterial damage arising from any use or non-use made of the information provided or from use of incorrect and incomplete information, unless due to proven intent or gross negligence on part of the authors.

The GRACE project is funded with support from the European Commission (FP7 Grant Agreement No 311957).

Introduction to GRACE

Motivation

Within the concept of a "knowledge-based (bio)economy," knowledge and information are increasingly being recognized as drivers for innovation, productivity and economic growth. In line with this development, policy decisions on new technologies and products resulting from their use, such as genetically modified (GM) crops, should be informed by comprehensive, science-based evidence on the potential impacts of these technologies and the resulting products on human and animal health, the environment, and society at large.

Based on the requirements laid down in the call (Call: FP7-KBBE-2012-6), the EU-funded project GRACE (GMO Risk Assessment and Communication of Evidence) aimed to increase the transparency and traceability of information about the potential risks and benefits associated with the deliberate release of GM crops. Two evidence synthesis concepts, namely systematic reviews and evidence maps, were applied to identify, analyse and communicate primary research data on potential impacts of GM crops and their products on human and animal health, the environment and socio-economic indicators in a transparent, reproducible and unbiased manner. Systematic reviews are evidence synthesis approaches which have become well established in medical science to inform evidence-based decision making. Their use is expanding to other disciplines to inform regulatory decision making, including food/feed safety assessment. Systematic reviews are based on a standardized and rigorous methodology to improve precision, minimize bias, and increase transparency – prerequisites for a robust synthesis of existing evidence.

Another task of GRACE was to test the design, execution and interpretation of rodent feeding trials (90-day and extended studies) and alternative studies with whole food/feed in order to provide recommendations on the appropriateness of these tools for the risk assessment of GM crops by considering the scientific strengths and limitations of the different approaches. The outcomes will be reviewed by the Commission by mid 2016 in order to reappraise the requirement to mandatorily perform a 90-day feeding trial with whole GM food/feed as demanded

by the Implementing Regulation (EU) No. 503/2013. The conclusions and recommendations drawn from data on animal feeding trials and alternative approaches have been discussed with stakeholders in October 2015 and are explicitly provided in the final chapter of this document. MON810 maize was chosen as a test material as several MON810 and near-isogenic varieties could be cultivated in Europe and several data sets from feeding trials with MON810 exist. It has to be underlined that GRACE is not expected to provide data for the reassessment of the safety profile of MON810 maize, but to explore the value of the different approaches for the risk assessment of whole GM food/feed.

Based on the transparency obligation of GRACE, active stakeholder involvement at both the planning and interpretation stages of our research activities was key to developing the scientific roadmap of the project and facilitating a broader reflection on the outcomes. The open-minded dialogue between GRACE and several stakeholder groups representing a broad spectrum of society led to an improved and more focused design of the research activities. We hereby acknowledge the valuable participation of stakeholders throughout the project period.

GRACE is further supporting open access to the data generated. This will be achieved by the open-access database CADIMA (Central Access Database for the Impact Assessment of crop genetic improvement technologies) which will document and support the evidence synthesis process, serve as a central access point for information related to GMO risk assessment and provide open access to research data. CADIMA will be continuously operated by the Julius Kühn-Institut (JKI) after the end of the GRACE project.

Though the project started in June 2012 to cover the 2012 cropping season, the signature of the Commission was received a month late, and this caused some delay in employing additional project staff during the summer. It put a burden on the project as a 3 months cost neutral prolongation was not granted by the Commission. Nevertheless, and thanks to all GRACE partners and participating stakeholders, the quality of the scientific output including an active stakeholder involvement was little affected.

Project changes

In the initial planning of GRACE, feeding trials with GM potatoes were anticipated. Those trials, however, had to be skipped due to the withdrawal of the provider (USDA) of GM potatoes.

While GRACE was expected to assess the potential added scientific value resulting from an extension of the feeding period beyond 90 days for the risk assessment of GM crops, the final decision to perform a trial with an exposure period of 1 year was made by weighing stakeholder comments, the potential scientific value of a possible extension and the temporal and financial constraints of the project.

Project organization

GRACE consists of ten work packages (WPs) which make up three pillars of project topics. The first pillar comprises two WPs and covers the evaluation of animal feeding trials and alternative approaches. A “Task Force” serves as a project body assuring the quality and harmonization of the working processes. Leading external scientists (currently Prof. Dr. Pablo Steinberg and Dr. Annette Pöting) reinforced the Task Force. The second pillar comprises four WPs providing a framework for evidence synthesis (one WP) and synthesising the evidence on potential impacts caused by the deliberate release of GM crops and products derived thereof on human and animal health, the environment and socio-economic indicators (3 WPs). The third pillar consists of two WPs and is responsible for the active involvement of stakeholders and the development and maintenance of the open-access database CADIMA. Communication activities and the project management are organized by two overarching WPs. The project management is supported by a Project Executive Committee (PEC), representatives of each WP, and an Advisory Board (AB) with external experts to independently advise on the implementation and progress. The GRACE consortium acknowledges the strong support provided by the Advisory Board (AB Minutes can be accessed via the GRACE website). The close collaboration between the different WPs and project management bodies and the flexibility of the project partners were a prerequisite for the success of the project especially in responding to time constraints and organizational challenges.

Stakeholder involvement and communication

Stakeholder involvement

For each of its two research work streams (evidence synthesis; animal feeding and alternative studies) GRACE employed a similar two-step stakeholder engagement approach: stakeholder consultations were held at both the planning stage of the GRACE research activities and at the results interpretation stage. Stakeholder comments had an impact on what was done in GRACE, how it was done, how the results were interpreted, which conclusions were drawn, and which recommendations were developed.

Openness and non-selectiveness: Invitations were circulated to a broad range of stakeholder organisations including, but not limited to, competent authorities at national and EU level, industry and business organisations as well as individual companies, civil society organisations, and researchers. Stakeholders interested in participating were accepted without selection. In addition to participating in several workshops, stakeholders were invited to comment on draft GRACE documents.

Transparency: Draft documents outlining research plans, research data, summaries of results, conclusions and recommendations were typically provided beforehand – in the case of animal feeding studies also including raw data. Stakeholder comments and discussions were documented in detailed reports including the responses of GRACE team members to written stakeholder comments. All reports are available at the GRACE website.

Multiple mechanisms for dialogue: Consultations included workshops, written comments and GRACE team responses, electronic questionnaires and phone interviews. Overall, five workshops and eight written consultations were conducted. On feeding and alternative studies a discussion forum for open access contributions was established in the scientific journal Archives of Toxicology in order to allow discussion to continue after publication of the GRACE results.

Tracking of stakeholder comments: All written stakeholder comments were addressed by the GRACE team and are presented in consultation reports. Each stakeholder participant, therefore, can track how his/her own comments were processed by the GRACE team. GRACE responses are also available to any interested third party via the GRACE website.

Over the entire project period 143 individual stakeholders from 19 EU Member States, the EU level, Switzerland, Norway, the USA and international organisations participated in GRACE stakeholder workshops and/or submitted comments in writing. They represent all major stakeholder groups and a broad spectrum of EU Member States. More than 1,100 written comments and questions were received. All discussions, stakeholder feedback and GRACE team responses are documented in 10 consultation reports.

Furthermore, stakeholders also provided feedback that assisted development of CADIMA services.

Following each major consultation step stakeholders were also consulted for feedback on the stakeholder engagement process itself.

As a result, several publications reporting on the stakeholder engagement process in GRACE are in preparation:

- Stakeholder engagement in regulatory science – the case of animal feeding studies in GMO risk assessment;
- Systematic reviews and evidence maps – a novel engagement issue for GMO stakeholders;
- Guidance on stakeholder engagement in evidence synthesis (joint publication with Collaboration for Environmental Evidence).

Communication

In line with the stakeholder involvement approach, the communication strategy of GRACE followed the principle of full transparency and unbiased reporting. The project promoted a recognisable open process, in which all relevant information is freely accessible. The project communicated objectives, study plans, interim results and final results of the feeding trials and alternative approaches via the project website and direct mailings to hundreds of stakeholders and it provided detailed information on how results were being generated, in particular on processes, methods used, and actors involved. A dedicated project website section openly documents all steps of the stakeholder engagement.

GRACE is also committed to answer any requests from journalists and to react to any criticism of the project in an open and transparent way. In such cases GRACE has always provided its responses and statements in the form of open letters presented on the GRACE website and announced via press releases to inform a broad range of stakeholder groups. For example, in October 2014, GRACE published the results of its first 90-day feeding studies with genetically modified MON810 maize. Shortly after, in a press release GRACE was suspected of having drawn the wrong conclusions from the trial data. In addition, it was claimed that some of the scientists involved in the study had links to industry and were therefore not impartial. GRACE has responded in detail to these accusations and to a related request of a journalist in a number of open letters and statements (the whole correspondence can be accessed via the GRACE website). In order to promote a scientific discussion about results and their interpretation, a discussion forum was established in the peer-reviewed journal Archives of Toxicology.

Achievements

Besides the originally planned potato feeding trials (reasons for their exclusion are described above), all tasks and experiments foreseen in the Description of Work were successfully completed.

Feeding studies and alternative approaches

Two different MON810 varieties, with respective near-isogenic controls, and four conventional maize varieties were explored as plant material. By using a diverse set of test approaches comprising two 90-day feeding trials, one longitudinal and metabolomics study with a duration of 90 days, a 1-year feeding trial, complementary *omics* analyses on animal tissues and plant material, and *in vitro* cell-based assays, GRACE provided a unique set of comparable data in order to draw conclusions on the appropriateness of design, execution and interpretation of rodent feeding trials and *in vitro* studies with whole food/feed for being considered in the risk assessment of GM crops. Also in this part, GRACE is setting the scene on active stakeholder involvement including transparent handling of study results and criticisms raised by certain stakeholder groups. Furthermore, all raw data will be made publicly available using CADIMA as a dissemination portal (or by a direct request to GRACE partners if the data volume exceeds the storage capacity of the database).

Peer-reviewed publications that have been produced so far:

- Enhancing the interpretation of statistical P values in toxicology studies: implementation of linear mixed models (LMMs) and standardized effect sizes (SESs), Archives of Toxicology, 2015, DOI:10.1007/s00204-015-1487-8
- Ninety-day oral toxicity studies on two genetically modified maize MON810 varieties in Wistar Han RCC rats (EU 7th Framework Programme project GRACE), Archives of Toxicology, 2014, DOI:10.1007/s00204-014-1374-8
- Facilitating a transparent and tailored scientific discussion about the added value of animal feeding trials as well as *in vitro* and in silico approaches with whole food/feed for the risk assessment of genetically modified plants, Archives of Toxicology, 2014, DOI:10.1007/s00204-014-1375-7

- Response to a report and press release by Bauer-Panskus and Then (2014) criticizing the presentation and interpretation of the results of recently published 90-day feeding studies with diets containing genetically modified MON810-maize varieties and their comparators (Zeljenková et al. 2014), Archives of Toxicology, 2015, DOI:10.1007/s00204-014-1429-x
- Small molecule and RNAi induced phenotype transition of expanded and primary colonic epithelial cells, Scientific reports 2015, DOI:10.1038/srep12681

Publications currently under preparation:

- *Omics* analysis of intestinal tissue samples from 90-day feeding trials
- Allergenicity and digestibility of GM maize
- Metabolomics analysis of 90-day feeding trials and the longitudinal and metabolomics study
- *Omics* analysis of plant material – maize materials – focus on comparison with the animal feeding trials
- *Omics* analysis of plant materials – potato materials – focus on one class model
- Results of the 1-year feeding study
- Results of the immunological assessment
- Proposal for criteria for the evaluation of the scientific quality of rat and mouse feeding trials with whole food/feed derived from genetically modified plants (joint publication between GRACE, G-TwYST and external experts)
- Statistical values and limitations of feeding studies to assess genetically modified crop safety – comparing the results of five toxicity studies

GRACE has identified the need to promote and explore synergistic effects between research projects to facilitate an exchange of expertise and data and to increase the comparability of results. Thus, a close collaboration between GRACE, the EU-funded project G-TwYST and the French project GMO90+ was established (a MoU was signed by the three project coordinators). All project coordinators have agreed to use CADIMA as a common dissemination platform.

Evidence synthesis

By applying systematic reviews and evidence maps to review questions that were discussed with and prioritized by active stakeholder participation, GRACE has endeavoured to provide rigorous and sound answers to the topics addressed, assured their relevance from a broader societal perspective and guaranteed a transparent and traceable evidence synthesis procedure. Due to the thorough evaluation of the methodological rigour of included studies that takes place within a systematic review, GRACE results have the potential to clarify uncertainties associated with the deliberate release of GM crops and to increase confidence in the available data. All review protocols and data will be made available via the open access database CADIMA and can readily be used by any user group including risk assessors, risk managers and the general public. Open discussion about critical appraisal criteria during the planning stage consultation and their subsequent application during the evidence synthesis process guided the harmonization of the review protocols in order to minimize the introduction of systematic errors that may affect the reliability of the results. Experience with applying evidence synthesis methods in GRACE has highlighted the value of review protocols as a communication tool, for facilitating a transparent dialogue about assessment criteria between parties involved in the evidence synthesis process.

The review topics covered within GRACE are as follows:

Potential environmental impacts:

- Effects of Bt maize on non-target animals
- Effects of GM herbicide tolerant crops on botanical diversity
- Effects of Bt crops on soil invertebrates
- Effects of Bt crops on soil microbial endpoints
- Susceptibility of lepidopteran/coleopteran maize pests to Bt proteins
- Inheritance of resistance alleles of lepidopteran/coleopteran maize pest species

Potential human and animal health impacts:

- Animal feeding studies with whole food/feed products derived from GM crops
- Changed allergic reaction to an allergenic crop after it has been genetically modified
- Key chemical crop constituents in GM crops compared to non-GM crops
- Toxicity studies in animals receiving newly expressed proteins from GM crops

Potential socio-economic impacts:

- Economic impacts of GM crops at the sectoral and macro level
- Trade impacts of GM crops
- Politics of GM crops
- Socio-economic impacts of GM crops worldwide

The review protocols can be accessed via CADIMA. As systematic reviews are relatively uncommon in environmental research we found only one scientific journal in the area of environmental conservation and management (Environmental Evidence) that has a formal system for the peer-review of systematic review or evidence map protocols.

Peer-reviewed protocols for GRACE reviews already available:

- Does the growing of Bt maize change abundance or ecological function of non-target animals compared to the growing of non-GM maize? A systematic review protocol. Environmental Evidence 2014, 3:7, DOI: 10.1186/2047-2382-3-7
- What are the effects of the cultivation of GM herbicide tolerant crops on botanical diversity? A systematic review protocol. Environmental Evidence 2014, 3:8, DOI: 10.1186/2047-2382-3-8
- Are population abundances and biomasses of soil invertebrates changed by Bt crops compared with conventional crops? A systematic review protocol. Environmental Evidence 2014, 3:10, DOI: 10.1186/2047-2382-3-10
- Are soil microbial endpoints changed by Bt crops compared with conventional crops? A systematic review protocol. Environmental Evidence, 2014, 3:11, DOI: 10.1186/2047-2382-3-11
- How susceptible are different lepidopteran/coleopteran maize pests to Bt-proteins: a systematic review protocol. Environmental Evidence 2014, 3:12, DOI: 10.1186/2047-2382-3-12
- What is the evidence on the inheritance of resistance alleles in populations of lepidopteran/ coleopteran maize pest species: a systematic map protocol. Environmental Evidence 2014, 3:13, DOI: 10.1186/2047-2382-3-13
- What are the socio-economic impacts of genetically modified crops worldwide? A systematic map protocol. Environmental Evidence 2014 3:24, DOI: 10.1186/2047-2382-3-24

Publications under preparation:

- For each review topic listed above, final manuscripts are under preparation. In the case of evidence maps for potential human and animal health impacts, these fall outside the scope of the journal Environmental Evidence so their protocols cannot be independently published and peer reviewed. Instead, these evidence maps will be submitted to an alternative scientific journal, and, if accepted after peer review, their associated protocols will (if possible) be provided as annexes to the core manuscripts.

- A general manuscript on the use of systematic reviews in socio-economic impact assessment and research on GMOs.

In order to harmonize the evidence synthesis process between different institutions, GRACE has established a close link with the Collaboration for Environmental Evidence (CEE), highly experienced in the conduct of systematic reviews and evidence maps in the area of environmental conservation. Based on this collaboration a Memorandum of Understanding (MoU) was signed between CEE and JKI.

Besides archiving completed evidence syntheses, CADIMA will provide online tools to assist review teams in the performance of systematic reviews and evidence maps. As agreed in the aforementioned MoU, JKI and CEE will both further promote the use of CADIMA and evidence synthesis. Since the value of active stakeholder involvement is broadly recognized within the evidence synthesis community, a stakeholder involvement group within CEE has been established, involving, among others, GRACE and CEE representatives.

GRACE activities further led to a proposal for good reviewing practice when synthesizing impact data on GMO, while a discussion on the adequacy of systematic reviews to inform the risk assessment and risk management process of GM crops by weighing associated benefits against potential limitations has been published in two papers:

- Developing a good practice for the review of evidence relevant to GMO risk assessment. GMOs in Integrated Plant Production, IOBC-WPRS Bulletin, Vol. 97, p.55-62, 2013
- Can systematic reviews inform GMO risk assessment and risk management? Frontiers in Bioengineering and Biotechnology, 2015, DOI: 10.3389/fbioe.2015.00113

Follow-up activities

Based on the results from the evidence map on animal feeding studies with whole food/feed products derived from GM crops, a systematic review will be performed on a specific subset of studies identified in the map and critical appraisal criteria being developed by a coordinated action between GRACE, G-TwYST and an external expert group will be applied to assess the reliability of the included studies. The CADIMA online tools will be used when performing the review and when making the results publicly available. A training workshop will be organized by JKI in April 2016 to familiarize interested users with the tools available in CADIMA. Furthermore, a contribution to the updated version of the CEE guidelines on the performance of systematic reviews will be provided, laying down the added value CADIMA provides when synthesizing evidence on specific review questions. Both activities will increase the broader awareness of CADIMA and might facilitate the decision of review teams to use CADIMA as a tool to support the conduct of future evidence syntheses.

Part I: Conclusions and recommendations on animal feeding studies and alternative approaches with regard to Article 12, Implementing Regulation (EU) No. 503/2013

The Implementing Regulation (EU) No. 503/2013 requires mandatory performance of 90-day rodent feeding trials for the authorisation of GM food and feed. Article 12 of the Regulation includes a review clause, stating that "the Commission shall review the requirement to perform 90-day feeding studies in rodents with whole genetically modified food/feed [...] on the basis of new scientific information". Furthermore, "the Commission shall in particular monitor the outcome of the research project called GRACE (GMO Risk Assessment and Communication of Evidence) under the 2012 work programme of the seventh Framework Programme for Research (FP7)". Thus, GRACE is expected to provide sound conclusions and recommendations on the adequacy of the approaches tested in the frame of GRACE for being considered during the regulatory approval process of GM crops in the future. In order to elaborate conclusions and recommendations, key questions or statements related to the scope of GRACE and answers based on the experiences and results achieved throughout the project are given. For clarity, the different feeding trials are named throughout this section according to the following scheme:

- Study A: 90-day study with Monsanto MON810 maize
- Study B: 90-day study with Pioneer MON810 maize
- Study C: 1-year study with Monsanto MON810 maize
- Study D: Longitudinal and metabolomics study with Monsanto MON810 maize
- Study E: Longitudinal and metabolomics study with Pioneer MON810 maize (Studies D and E were originally performed as one single study and were subdivided into two studies during the statistical analysis process)

1. Design, performance and interpretation of animal feeding studies with whole food/feed for GMO risk assessment

1.1. Which conclusions can be drawn from the 90-day studies performed within GRACE with regard to the design, performance and interpretation of results?

Distinction between statistically significant differences and toxicologically relevant differences

- When interpreting the results from animal feeding trials conducted in the course of GRACE, all statistically significant differences between groups have been evaluated with regard to their toxicological relevance. The varying patterns of significance in the different studies as well as discussions arising from the publication of the results from Studies A and B highlighted the importance of this distinction: i.e. single statistically significant difference is not necessarily indicative of organ or tissue specific toxicity, which generally becomes evident through changes in several related parameters.

Value of historical control data when interpreting study results: Should only data sets generated by the same laboratory using the same type of diets be taken into account?

- Historical control data provide information on the typical variability regarding specific endpoints within a given setting and hence help interpreting the study results and putting them into context. Based on the experiences gained by GRACE during Studies A and B, historical control data support the reasoning about a potential toxicological relevance of statistically significant differences observed when compared to the concurrent control.

- The comparison of the test group(s) with the concurrent control group is always the most important consideration, but appropriate historical control data can help interpret results in a number of situations. If no such data exist in the testing facility – as it was the case before the start of the Studies A and B – additional control groups should be included in the study in order to allow for an assessment of variability.
- Ideally, historical control data should be generated by the test facility performing the trial within an appropriate time period, using rats of the same age and strain and the same type of diet (OECD, 2002; OECD, 2012) to estimate the lab-specific variability of endpoints. Further elaboration on the use of historical control data in the context of whole food/feed testing will be performed in the EU-funded project G-TwYST.

Is the use of the standardized effects size (SES) approach a step forward in the statistical analysis of the data obtained in the feeding trials and does the way of data presentation facilitate the evaluation of findings?

- Based on the experiences gathered by GRACE, the SES approach eases the interpretation and contextualization of the gathered results by providing an overall picture of potential group differences and the associated magnitude of difference. Predetermined deviations of toxicological concern can be co-displayed within the SES graphs.

Do the outcomes of feeding trials performed within GRACE justify any preference for an extension of the exposure period from 90 days to 1 year?

- Studies A and B did not reveal any scientific trigger for an extension of the feeding period.
- Data gathered in the course of Study C (1 year) concur with the conclusions made after 90 days (Studies A and B) that administration of maize MON810 to rats did not show adverse effects.
- The extended study conducted in GRACE did not provide relevant additional information compared with the 90-day studies.

1.2. Would the inclusion of additional endpoints (additional to the endpoints as stipulated in OECD guideline 408) increase the scientific value of 90-day feeding studies with whole GM food/feed?

General issues

Would a targeted and testable hypothesis be needed when deciding about the inclusion of additional specific endpoints?

- A targeted and testable hypothesis would be necessary to inform the decision about the choice of specific endpoints and associated parameters to be included in the feeding trial.
- When planning and interpreting the study, the biological variation within the tested parameter should be taken into account.

Immunotoxicity

What options are considered within GRACE to include an assessment of immunogenicity/allergenicity or immunosuppression of GM plants in the frame of animal feeding trials with whole food/feed within the risk assessment of GM crops?

- Immunotoxicity testing can provide extra information for safety assessment, especially when new proteins that do not currently form part of the human diet and raise safety concerns based on the initial *in vitro* and *in silico* analyses are incorporated into food/feed. At this moment, different *in vivo* tests using animal models have been developed, but not yet validated for regulatory purposes. Allergenicity assessment as recommended by EFSA applies a "weight-of-evidence" approach including in silico and *in vitro* testing. Within GRACE the results of the recommended *in vitro* studies (on maize) were related to those obtained *in vivo*.

- In the GRACE experiments, targeted serum screening was applied for the assessment of allergenicity and immunogenicity of GM vs. near-isogenic control maize and commercial varieties. Immunosuppression was examined by phenotypic analysis of leukocytes in rat tissues, and three *in vitro* immune function assays (proliferative activity of lymphocytes upon toxin/protein/mitogen stimulation, production of cytokines, and phagocytic activity and respiratory burst of leukocytes). Validated standard operating procedures and routine applications are yet not available. High inter-individual variability in animal immune responses occurred, as expected, which might limit the applicability of the assays (similar variability in the immune response is observed in the human population).

Omics on animal tissues

What may be the specific role for transcriptomics, proteomics and metabolomics in the process of risk assessment?

- In case of *targeted* approaches, the integration of *omics* screening of potential target organs of toxicity provides a basis for the identification of causal molecular patterns associated with toxicity. The identification of pathways of toxicity may unravel mechanistic details that enhance the regulator's confidence in classical endpoints by providing a mechanistic basis for decision-making.
- In the GRACE project, intestinal tissues from rats, which had primarily been exposed to the ingested feed materials, were analyzed. These tissues have important functions in terms of immunology, biotransformation, and signaling responses to xenobiotics. Bioinformatics analysis of the transcript*omics* data (from studies A and B; › 25.000 genes) revealed a clear temporal pattern in expression of genes being related to the sampling time.

- Perturbations of pathways of toxicity have not been detected, underlining the study results revealed by classical OECD endpoints.
- The longitudinal study design has the potential to detect progressive changes when compared to single endpoint measurements: no such changes in the longitudinal metabolomic study in the GRACE project (studies D and E) were detected; this reinforces the finding that no toxic effect could be detected during the sub-chronic studies.
- GRACE experimental data underline that an application of transcriptomics/proteomics approaches without a targeted and testable hypothesis as well as adequate controls did neither facilitate hypothesis generation nor did it reveal unintended effects of feeding MON810 maize. Unintended effects were also not detected in metabolomic analyses with sera from the studies A and B as well as from studies D and E.
- GRACE data indicated that *omics* approaches detect the coordinated homeostasis of the examined tissue(s) which in turn may facilitate the detection of nutritional or toxicological perturbations. Variations in experimentally controlled and in non-controlled factors were trackable (e.g. time of sampling, linked with circadian rhythms, heterogeneity of sampling related to the cellular composition or anatomic sub-sites, etc.). Therefore, the experimental design should be based on clear-cut questions and optimized for *omics* approaches and a careful evaluation of the available data must be ensured to prevent misinterpretations.

2. Analysis of plant material

2.1 What is the perspective of non-targeted *omics* approaches within the overall risk assessment of GM crops (also in comparison to animal feeding studies with whole food/feed)?

Could *omics* approaches provide an added scientific value when compared to the information gathered by the targeted compositional analysis in order to assess GM crop safety (also in comparison to animal feeding trials with whole food/feed)?

- GRACE data have shown that the comparative safety assessment (currently the concept applied in the Implementation Regulation 503/2013 based on earlier EFSA guidelines) can also be adopted for *omics* data e.g. using the one-class model approach: the GM variety can be compared to its closest conventional comparator, as well as to a range of conventional varieties. Standardized and validated test procedures are currently not available.
- The GRACE data have shown that the one-class model classifies mycotoxin-contaminated maize samples as outside of the one 'safe' class (additionally sampled in Spain, not related to the maize materials that have been fed to the test animals). As no further analyses on possible contaminants were performed on this sample, no indications about possible toxicity can be derived but the results would provide a scientific basis for further analysis. Similarly, the one-class model classifies experimental potato varieties that are fit for human consumption but genetically more distant from the lines that are currently consumed, in almost all cases as outside of the one 'safe' class (indicating that the one-class model represents a conservative approach). All maize varieties fed to the test animals in the course of GRACE were classified by the one-class model as inside of the one 'safe' class. Due to time limitations, the maize harvest included in the diet in Study C was not analyzed by using the one-class model.

- Furthermore, GRACE *omics* data have shown that elements with a foreign origin as e.g. the Cry1Ab gene and its expressed product in the MON810 maize can readily be identified by following this non-targeted approach.
- Based on these observations, it can be stated that *omics* data as generated by GRACE provide qualitatively structured details of the plant material which facilitates a non-targeted "safety" evaluation. Thereby it provides a better basis for the decision on the scientific rationale to frame the subsequent risk assessment steps, which may include the performance of an animal feeding trial with the plant-derived whole food/feed.

3. *In vitro* cell and tissue cultures

3.1 What would be the perspective of cell culture systems within the overall risk assessment?

From a technical point of view – is it possible to perform cell culture studies using whole maize/feed materials or extracts from it? Are the levels of exposure to newly expressed proteins *in vitro* comparable to the levels *in vivo*?

- In the GRACE project, we assessed and optimized plant extracts for application in primary and permanent intestinal cell cultures (pig, rat). Aqueous extracts were prepared using basal media of the respective cell cultures, and dose testings were performed for optimization. Quantification of Cry1Ab protein in maize extracts revealed that exposure levels achieved *in vitro* can be manifold higher than published concentrations in *in vivo* digesta.

What would be the requirements for *in vitro* toxicity assay validation particularly for whole plant/feed materials? What would be adequate positive controls?

- The validation of novel *in vitro* tests requires the comparison to available *in vivo* data. Therefore, targeted feeding trials with whole food/feed with toxicological potential are needed to prove the validity of the developed test. Currently, there are no *in vitro* methods for complex endpoints like repeated dose systemic toxicity and the development of such assays will require major scientific advances; therefore, *in vitro* assays can be developed for selected endpoints of toxicity and contextual positive controls should be selected.

4. General considerations: Animal feeding studies

4.1. Can 90-day or extended feeding studies with whole GM food/feed provide an added scientific value for the risk assessment of GM crops?

- GRACE data did not provide any indication that the performance of 90-day feeding studies (following OECD or EFSA guidelines and current practice) with whole food/feed would provide additional information on the safety of maize MON810 when compared to the compositional comparison of the GM line and its closest conventional comparator in terms of an initial comparative safety assessment. These findings are in line with the EFSA Guidance Document (EFSA 2011) that forms the basis of the current Implementing Regulation 503/2013.
- The data generated by GRACE showed that non-targeted feeding studies may generate outcomes at the level of the variability of the lab i.e. generating significant differences randomly. Such results do not inform the risk assessment nor do they increase the confidence in the data provided.
- GRACE data support the scientific reasoning that only in case a trigger is available from the initial molecular, compositional, phenotypic and/or agronomic analyses, feeding trials with whole food/feed may provide an added scientific value for the risk assessment of GM crops (EFSA, 2011).
- If safety concerns are raised during the molecular, compositional, phenotypic and/or agronomic analyses, a feeding trial might be considered, provided that a targeted hypothesis can be developed to tailor the study design to the posed safety concern.
- The need to provide nutritionally balanced diets determines the maximum inclusion rate of the plant material to be tested and thus restricts the exposure level of the animals to the respective food/feed.

- Linked to this, the expected magnitude of a distinctly identified potential effect should be included into the test hypothesis and trigger the decision whether a feeding study should/could be performed to achieve a clear test response.
- The study design should follow the test hypothesis and should provide clear test results. A standard approach may not be the primary choice to achieve this.
- The general design of the feeding trials is described in the EFSA Guidance on conducting repeated-dose 90-day oral toxicity studies in rodents on whole food/feed and by the corresponding explanatory statement. Based on the experiences gathered within GRACE it should be highlighted that:
  - Adequate historical control data should be generated in the test lab to improve statistical planning and support valid conclusions.
  - A targeted and testable hypothesis would promote the performance of a meaningful power analysis and improve certainty of results achieved.
  - Randomized sampling is highly recommended especially if additional end-points – e.g. related to *omics* – are to be recorded.

5. RRR (Replacement, Reduction, Refinement)

5.1 Under which conditions could the consideration of feeding studies with whole GM food/feed within the risk assessment of GM crops be justified in the light of the RRR approach?

Is a mandatory performance of feeding studies with whole GM food/feed justified in the light of the RRR approach?

- Due to the intrinsic limitations of a feeding trial with whole food/feed, a mandatory performance cannot be justified in the light of the RRR approach based on the available science.
- This is further strengthened by the vast amount of animals that were sacrificed in the course of GRACE [Studies A and B (90 days): 160 animals/study, Studies D and E (90 days): 120 animals/study, Study C (1 year): 160, in total: 720].
- *Omics* approaches on plant material may inform the development of a targeted hypothesis in the future in order to scientifically justify the performance of feeding trials with whole food/feed and to target the study design to the posed safety concern.
- The expected magnitude of a distinctly identified potential effect should be included into the test hypothesis and guide the decision whether a feeding study should/could be performed to achieve a valuable information gain.

Part II: Conclusions and recommendations on the use of systematic reviews and evidence maps when summarizing and evaluating GMO impact data

Systematic reviews are evidence synthesis approaches which have become well established in medical science to support evidence-based decision making. Recently, their use is expanding to other disciplines to inform decisions including GM food/feed safety assessment. GRACE applied Systematic Reviews and Evidence Maps for research questions in the scope of the impact assessment of GM crops. The conducted evidence syntheses included environmental and socio-economic impacts in addition to health impacts.

Systematic reviews are based on a standardized, transparent and rigorous methodology to minimize bias and optimize precision. Thus, systematic reviews could be especially valuable for synthesizing evidence relating to contentious topics for which stakeholders may hold differing views. Evidence maps are similar to some steps of systematic reviews except that they systematically explore the evidence available within a specific research area, without attempting to quantitatively synthesize the identified evidence.

In the following, we present conclusions and recommendations based on the experiences and results obtained during the GRACE project.

1. Are systematic reviews and evidence maps appropriate approaches when summarizing GMO impact data?

Weighing up the appropriateness of systematic reviews and evidence maps, the following potential benefits and limitations have to be considered.

Potential benefits:

Related to both evidence synthesis approaches

- Minimizing bias by the elaboration of a review protocol and the impartial application of assessment criteria
- Increasing transparency and traceability by assuring thorough documentation of the review process
- Facilitating stakeholder involvement (e.g., by discussion of the review protocol)
- Facilitating updating by following a standardized and thoroughly recorded procedure
- Facilitating a transparent communication of assessment details by means of the review report and increasing the traceability of review conclusions

Related to systematic reviews only

- Increasing precision by means of a quantitative data synthesis, e.g., via meta-analysis, thereby facilitating the clarification of uncertainties
- Thus, systematic reviews provide a robust answer to a specific question and a defensible input to evidence-based discussions.

Related to evidence maps only

- Broader questions can be addressed. Nevertheless it has to be assured that the question is framed in such a way that an appropriate search strategy and selection criteria can be developed, leading to a workable amount of reports.
- Can provide an overview of the extent, range and nature of extensive research activities in a particular field.
- Thus, evidence maps facilitate a comprehensive analysis of the structure of available data and their sources, help to identify knowledge gaps and provide a basis for identifying priority areas for systematic reviews.

Potential limitations:

Related to both evidence synthesis approaches

- Systematic reviews and evidence maps can be resource intensive.
- Where answers are required for many research questions, prioritization of questions may be necessary.
- Rapid answers are not possible.

Related to systematic reviews only

- A focused research question has to be developed.
- Sufficient primary research data have to be available to allow a useful integration of data.

Related to evidence maps only

- Evidence maps do not provide a quantitative data synthesis but rather characterize the underlying evidence base.

Due to these possible limitations, the appropriateness of systematic reviews and evidence maps would depend on the specific topic and question under assessment and a decision for or against their performance has to be made on a case-by-case basis.

2. How do systematic reviews and evidence maps support the communication of GMO impact study outcomes?

- Based on the experiences of GRACE, systematic reviews and evidence maps facilitate the communication of GMO impact data by
  - allowing a transparent discussion of assessment criteria by means of the review protocol
  - clarifying exactly which evidence has (or has not) been included
  - assuring the traceability of review conclusions
- Systematic reviews allow to quantitatively reconsidering study designs and methodologies including appropriateness of measurement endpoints. Thereby, they can provide feedback how to raise the study quality.

3. How can systematic reviews and evidence maps inform GMO risk assessment and risk management?

The following information is adapted from "Can systematic reviews inform GMO risk assessment and risk management? Frontiers in Bioengineering and Biotechnology, 2015, DOI: 10.3389/fbioe.2015.00113"

Evidence synthesis and GMO risk assessment

- During problem formulation and based on a conceptual model, systematic reviews could support a more rigorous evaluation of relevant parameters/ variables by providing defensible (quantitative) answers to support decisions when framing the scope of subsequent risk assessment steps.
- During hazard and exposure characterization, systematic reviews could provide valid quantitative estimates regarding the intensity or likelihood of a hazard.
- During the development of risk management strategies, systematic reviews may provide robust statements about factors which can influence the efficiency of management strategies.
- The availability of primary research data on new or rarely studied events could be a major limitation restricting the use of systematic reviews within the regulatory approval process of GMOs. Thus, systematic reviews might only be feasible on a case-by-case basis where the available evidence base would justify their conduct.
- The Implementing Regulation (EU) No 503/2013 on applications for authorization of genetically modified food and feed requests the applicant to "include a systematic review [....] on potential effects on human and animal health of the genetically modified food and feed covered by the application". GRACE experiences show that the posed question would be too broad to be answered by a single systematic review, which requires the specification of a well framed and focused question. Hence, clarification on the intended meaning of this statutory requirement is urgently needed.

- During case-specific monitoring, systematic reviews could facilitate the integration and weighing of new studies by providing a consistent, transparent and robust evaluation scheme that is readily updatable.
- Evidence maps may provide an overview of ongoing research activities falling within the scope of the general surveillance (i.e. the detection of unintended effects that were not anticipated in the risk assessment). Even though broader questions can be addressed by an evidence map than by a systematic review, care has to be taken that the question is not so broad that the evidence synthesis becomes difficult to manage. As the scope of the general surveillance would be too broad to be covered by one evidence map, specific questions would need to be identified and prioritized.
- In order to determine the scientific justification of evoked safeguard clauses or further emergency measures, systematic reviews might promote the weighing of new information and the assessment of its impact on previous risk/safety conclusions (e.g. via sensitivity analysis), provided that the new data are within the scope of an existing systematic review already used to inform GMO risk assessment and are available in the public domain. However, updating and reanalyzing a systematic review may be too time consuming, hindering the provision of a timely answer which may be required if there are concerns about imminent harm.

Taken together, systematic reviews and evidence maps may provide added value when informing different risk assessment or risk management steps about GMO impacts. However, due to the aforementioned limitations, a decision about their conduct has to be made on a case-by-case basis and thus a mandatory systematic review as required by the Implementing Regulation (EU) No 503/2013 would not be feasible.

4. General conclusions from systematic reviews and evidence maps performed within GRACE

In order not to pre-empt the publication of GRACE results, only general conclusions are provided at this stage. Detailed conclusions drawn from GRACE systematic reviews and evidence maps will be provided in the respective manuscripts upon publication.

Evidence synthesis on potential environmental impacts

- GRACE reviews confirm the conclusions of previous risk assessments of Bt and HT crops with regard to field impacts on the evaluated groups of organisms.
- They provide complimentary scientific information that may inform risk assessors and managers, and those involved in environmental monitoring and integrated pest management.
- They provide weight of evidence information that may inform those making policy and decisions.

Evidence synthesis on potential health impacts

- Evidence maps can inform both risk assessment and risk management communities.
- The drafted evidence maps on the four health-related topics (toxicity of newly expressed proteins and whole foods/feeds, allergenicity, composition) showed that publications straddle a wide range of crops, newly introduced traits, experimental animal species and other experimental models and parameters employed.

- The references retrieved through the search actions but de-selected during further selection and extraction, may also provide interesting insights, such as the observation that a high number of studies have been published on the production and characteristics of oral vaccines and other pharmaceuticals in GM crops (used as "plant factories"), and the seemingly increasing number of publications by Chinese and other Asian authors in recent years (e.g., in non-English literature).

Evidence synthesis on potential socio-economic impacts

- Evidence gaps and areas for further research have been identified in terms of geographical focus and research fields (e.g. supply chain, environmental economics, food security, distribution impact of trade-related measures among actors).
- GRACE team created an extensive and unique database on socio-economic impacts of GM crops that serves as a solid foundation and guide for future research.
- Preliminary conclusions revealed that the introduction of GM crops does matter in terms of aggregate welfare change and that there are mixed results on trade-related impacts of GM crops, some of which are in line with previous empirical findings.
- A number of factors were identified explaining heterogeneous impacts across the selected studies.
- Evidence synthesis approaches are, if feasible, particularly valuable tools when informing decision-making.
- Relevant statistics are often not reported and this might affect the way a quantitative data synthesis is performed. For future research on socio-economic impacts of GM crops it is recommended that more comprehensive statistical information on the data used should be provided.

5. Further issues to be considered

- Based on the GRACE experiences, review teams should be trained in systematic review and evidence map methodologies before initiating the respective evidence synthesis.
- A considerable number of studies in the field of GMO impact assessment have been conducted for regulatory purposes and have never been published. Such studies are typically included in applications for market releases. In the EU they can only be accessed by regulatory bodies and thus their identification and inclusion in publicly accessible systematic reviews or evidence maps would face considerable hurdles. Relevant studies would need to be identified by gaining read access to an application (from EFSA), and then permission for the further use of study data must be obtained from the data owner. Therefore, a considerable body of evidence would likely be excluded from evidence syntheses.
- It is good practice that both the review protocol and the final review report undergo an independent peer-review process. Based on the GRACE experiences, there is the need to establish such an infrastructure (e.g. academic journals) covering all areas of GMO impact research.

Annex 1: List of GRACE partners

Participant no.	Participant organisation name	Country
1 (Coordinator)	Julius Kühn-Institut (JKI)	Germany
2	Agrobioinstitute (ABI)	Bulgaria
3	Aarhus Universitet (AU)	Denmark
4	Centre for European Policy Studies (CEPS)	Belgium
5	Centre de Recerca Agrigenòmica Consorci CSIC-IRTA-UAB (CRAG)	Spain
6	Agroscope	Switzerland
7	Freie Universität Berlin (FUB)	Germany
8	Genius GmbH (GENIUS)	Germany
9	International Centre for Genetic Engineering and Biotechnology (ICGEB)	Italy
10	Interdisziplinäres Forschungszentrum für Technik, Arbeit und Kultur (IFZ)	Austria
11	Institut National de la Recherche Agronomique (INRA)	France
12	PERSEUS BVBA (PERSEUS)	Belgium
13	Stichting Dienst Landbouwkundig Onderzoek (SDLO-RIKILT)	Netherlands
14	Sweet Environmental Consultant (SEC)	UK
15	Slovenska Zdravotnicka Univerzita v Bratislave (SZU)	Slovakia
16	Technische Universität München (TUM)	Germany

17	Council for Scientific and Industrial Research (CSIR)	South Africa
18 (retired in Q1/2013)	United States Department of Agriculture Agricultural Research Service (USDA-ARS)	USA
19	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	Germany
20	BioMath GmbH	Germany
Third Party	University of Girona	Spain

Annex 2:
Advisory Board and Task Force Members

Advisory Board members

Yaroslav Blume, Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine (Kyjiw, Ukraine)

Didier Breyer, Scientific Institute of Public Health (Brussels, Belgium)

Yann Devos, European Food Safety Authority, GMO Panel (Parma, Italy)

Geoff Frampton, Southampton Health Technology Assessments Centre (Southampton, UK)

Kaja Kantorska, European Commission, Directorate General Health and Consumers (Brussels, Belgium)

Annette Poeting, Federal Institute for Risk Assessment (Berlin, Germany)

Huib de Vriend, LIS Consult (Driebergen, The Netherlands)

Task Force members

Karine Adel-Patient, Institut National de la Recherche Agronomique (Gif-sur-Yvette cedex, France)

Ralf Einspanier, Universität Berlin (Berlin, Germany)

Gijs Kleter, RIKILT Wageningen UR (Wageningen, The Netherlands)

Christian Kohl, Julius Kühn-Institut (Quedlinburg, Germany)

Esther Kok, RIKILT Wageningen UR (Wageningen, The Netherlands)

Maria Pla, Universitat de Girona (Girona, Spain)

Annette Pöting, Federal Institute for Risk Assessment (Berlin, Germany; external expert)

Joachim Schiemann, Julius Kühn-Institut (Quedlinburg, Germany)

Kerstin Schmidt, BioMath GmbH (Rostock, Germany)

Pablo Steinberg, University of Veterinary Medicine Hannover (Hannover, Germany; external expert)

Ralf Wilhelm, Julius Kühn-Institut (Quedlinburg, Germany)

Dagmar Zeljenkova, Slovak Medical University (Bratislava, Slovakia)