GRACE
GMO Risk Assessment and Communication of Evidence

European Commission 7th Framework Project
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OPEN LETTER

to

DI Dr. Eva-Claudia Lang (Federal Ministry of Health, Austria) in response to her letter of 18 January 2016 addressed to Dr. Tomasz Calikowski (EC – DG Research and Innovation):

"AUSTRIAN STATEMENT CONCERNING THE FINAL REPORT OF GRACE / LETTER TO EC"

and enclosed

"COMMENTS ON THE FINAL REPORT OF THE GRACE PROJECT"

of the authors Dr. Werner Brüller, DI Walter Stepanek and Mag. Markus Wögerbauer (Austrian Agency for Health and Food Safety)

Dear DI Dr. Eva-Claudia Lang,

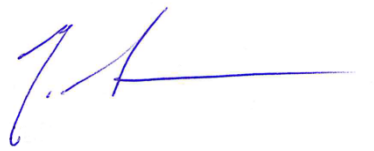
This letter is sent to you in response to your letter dated 18 January 2016 to Dr. Tomasz Calikowski (EC – DG Research and Innovation). In your letter you stated the following:
"Stakeholder consultations have been regarded as a core element of the GRACE project, covered even by a dedicated work package. The opportunity to engage in an open-minded dialogue between the GRACE consortium and several stakeholders is mirrored in the "Conclusions and recommendations" of the GRACE consortium and certainly welcomed. But it is astonishing that none of the concerns expressed by the stakeholders have been picked up and discussed in this final report. That's why Austria now takes the opportunity and expresses its concerns regarding this study directly to the responsible General Directorates of EC."

In your letter you enclosed a scientific opinion of your national experts concerning the final report of the GRACE-study. You will find in the Annex a detailed response from the GRACE team to comments raised by Dr. Werner Brüller, DI Walter Stepanek and Mag. Markus Wögerbauer.

The research within GRACE was carried out according to established scientific standards and under conditions of well-documented quality control and good practices. Additionally, the GRACE consortium attached great value to dialogue and transparency, among others by involving stakeholders during various stages of the research design and result interpretation (see: http://www.grace-fp7.eu/). Data obtained during the experiments have been / will be made publicly available, and allow for critical appraisal.

We would like to draw your attention to the fact that Archives of Toxicology offers a platform for scientific discussion of the GRACE results. All those interested are invited to submit comments on current and upcoming publications in the form of letters to the editor of Archives of Toxicology (see Arch Toxicol. 2014; 88(12): 2067–2069). These contributions will be published together with responses from the research projects. We would be delighted if your national experts would play an active role in this discussion. A direct, transparent and interactive scientific discussion of the arguments put forward is, we believe, extremely important and constructive. We hope that our communications in future can be held in the spirit of dialogue and transparency fostered by the GRACE project.

Yours sincerely,



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Encl: Annex with detailed response to comments raised by Dr. Werner Brüller, DI Walter Stepanek and Mag. Markus Wögerbauer

ANNEX

Response to Comments by Dr. Werner Brüller, DI Walter Stepanek, and Mag. Markus Wögerbauer (Austrian Agency for Health and Food Safety, Division for Data, Statistics & Risk Assessment) on the final report of the GRACE project: **„Conclusions and recommendations on animal feeding trials and alternative approaches and on the use of systematic reviews and evidence maps for GMO impact assessment“**

Comments on the final report of the GRACE project:
„Conclusions and recommendations on animal feeding trials and alternative approaches and on the use of systematic reviews and evidence maps for GMO impact assessment“ (1)

1) In the 90-day feeding studies, the specific values (especially significant differences) are to be compared between the groups within the actual test system which should be homogenous and underlying the same outer conditions. This has to be predominantly relevant. The comparison against a historical control (or conventional groups included in the test system) weakens the statistical significance because of increasing the statistical variance.

Answer:

It has to be pointed out that: 1) in all five feeding trials performed by the GRACE consortium the results obtained for each endpoint in the groups fed the genetically modified (GM) maize were specifically compared with the corresponding control group; 2) the comparison “group fed the GM maize vs. control group” was the basis for the discussion of all results in all five feeding trials. The relevance of the study-specific control data was emphasized in the GRACE conclusions and recommendations ("The comparison of the test group(s) with the concurrent control group is always the most important consideration", lines 1-2 on page 22 in ref. [1]). However, to contextualize the results it was necessary and legitimate to mention in dedicated cases that certain effects, which could have been ascribed to the GM maize, were also observed with a conventional maize variety tested in the same study. Consequently, in such cases it could be concluded that the observed differences likely reflect normal variation and were not specifically related to the genetic event.

When analyzing the results of the first two 90-day feeding trials (studies A and B) performed by the GRACE consortium, in a limited number of cases and in the absence of own adequate historical control data at the Slovak Medical University (where the feeding trials were performed) we alluded to historical control data collected by Harlan. This comparison was never taken as a stand-alone argument, but was used in certain cases as a complementary argument to support our conclusions on whether an effect was or was not specifically related to the genetic event. In the later feeding trails C, D and E [C = 1-year trial, manuscript submitted; D and E = 90-day longitudinal metabolomics trials, manuscript in preparation], we never made use of the Harlan historical control database to interpret the obtained results since in the frame of the GRACE project the animal housing facility at the Slovak Medical University produced its own historical control data (Schmidt et al. [2016],

manuscript in preparation). The GRACE conclusions and recommendations also emphasized the need of appropriate historical control data and how these data should be generated to minimize the statistical variance within these data ("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)", lines 7-9 on page 22 in ref. [1]).

2) There have been numerous findings which might have an impact on the trustability or might be the first step of detrimental effects for the time being only slightly apparent because of the relatively short duration and very low dosages:

Regarding the results of both 90-day feeding studies there are numerous significant differences of the verum groups compared to the control. They become substantially apparent when the values also differ from the two conventional groups, having the same trend as in comparison to the control.

Some examples - pars pro toto - are given in the following:

Trial A

Glucose values are statistically significantly increased in male verum rats (in this case also higher than both conventional group values), with the same trend in female verum rats. With male rats, creatinine is significantly lowered as against the control, with the same trend compared to conventional groups.

Trial B

Red blood cell counts are significantly elevated, both in male and female verum rats, in both cases the same trend can be seen against both conventional groups.

In female verum rats, neutrophils are dose-dependently decreased (neutropenia), also against both conventional groups. In the same species, monocytes are dose-dependently increased (monocytosis), same at least compared to one conventional group.

In female verum rats, thymus weight is significantly dose-dependently increased, also as against conventional groups.

Answer:

1) The Austrian colleagues argue that findings regarding various parameters (i.e. glucose, creatinine, red blood cell counts, percentage of neutrophils, percentage of monocytes and thymus weight) “might be the first step of detrimental effects for the time being only slightly apparent because of the relatively short duration and very low dosages”. It must be said that the above-mentioned parameters were not only measured in male and female rats fed the diets containing 11% or 33% MON810 maize for 90 days in the feeding trials A and B to which the Austrian colleagues refer to, but also after 90 days and after 1 year in the 1-year trial C there was no difference in any of the above-mentioned parameters between rats having been fed the control, the 11% MON810 or 33% MON810 diet. The complete data set (as means ± standard deviation of each endpoint) was available to Dr. W. Brüller and DI W. Stepanek, since they attended the

GRACE Stakeholder Meeting in October 2015 (see also Answer to Comment 9 explaining access to data generated by GRACE animal feeding studies). Unfortunately, this data set was not taken into account in their Comment.

2) The relevance of the changes in the blood glucose levels (and the pancreas weight) of feeding trials A and/or B was assessed by Zeljenková et al. (Arch. Toxicol. 88: 2289-314, 2014) and Steinberg (Arch Toxicol. 89: 137-139, 2015) and reassessed when analyzing the data of the 1-year study (Zeljenková et al., manuscript submitted) as follows:

   A number of facts support the conclusion that the decrease in pancreas weight changes and in the blood glucose levels observed in the 90-day feeding trials A and B are not related to the feeding of the GMO-containing diets:

   I) The blood glucose levels were not altered in female rats fed the GMO diets in the feeding trial A as well as in male and female rats fed the GMO diets in the feeding trial B.

   II) The measured blood glucose values in male rats fed the 11% and 33% GMO diet in the feeding trial A were within or close to the ranges of the groups of male rats fed the conventional maize varieties.

   III) The increase in the blood glucose levels of male rats fed the 11% and 33% GMO diet in the feeding trial A was not dose-dependent.

   IV) The decrease in the pancreas weight of male rats fed the 11% and 33% GMO diet in the feeding trial A was not dose-dependent.

   V) The decrease in the pancreas weight was also observed with the two conventional maize varieties used in the feeding trial A, whereby the decrease only reached statistical significance in the case of the conventional 2 variety.

   VI) Neither the blood glucose levels nor the relative pancreas weight were altered after feeding the diet containing 11% or 33% MON810 for 1 year to male and female rats if compared to the values of the corresponding control group.

   The Austrian colleagues point out to the decrease in the blood creatinine levels in male rats in trial A. The blood creatinine level is a parameter reflecting renal function and is increased in case of a renal dysfunction like renal insufficiency or as a consequence of renal toxicity. The fact that the creatinine blood level is lower in rats fed the GM maize than in rats fed the control (i.e. the near-isogenic non-GM) maize has no pathophysiological significance at all and is in line with the view that there is no kidney toxicity due to the ingestion of MON810. This conclusion is supported by the fact that in the 1-year feeding trial no differences in the blood creatinine level between the control male and the MON810-fed male rats were observed.

   The Austrian colleagues point out to the increase in the red blood cell counts in male and female rats in trial B. The following facts support the conclusion that the increase in the red blood cell counts in the feeding trial B is not related to the feeding of the GMO-containing diets:

I) The increases in the red blood cell counts were minimal (about 4% in the male rats and about 3% in the female rats) and not dose-dependent, i.e. there were no differences in the red cell blood counts between rats fed the 11% MON810 diet and those fed the 33% MON810 diet.

II) No differences were observed in the red blood cell counts of male and female rats fed the 11 and 33% GMO diets in the 90-day feeding trials A, D and E.

III) In the 1-year feeding trial C no differences in the red blood cell counts between the control and the MON810-fed rats (in the case of males as well as females) were observed.

The Austrian colleagues point out to the decrease in the percentage of neutrophils in female rats in trial B. The following facts support the conclusion that the decrease in the percentage of neutrophils in the feeding trial B is not related to the feeding of the GMO-containing diets:

I) The percentage of neutrophils remained unaffected in female rats fed the 11% MON810 and the 33% MON810 diets in the 90-day feeding trials A and D.

II) The percentage of neutrophils was significantly increased in female rats fed the 11% MON810 diet and remained unchanged in female rats fed the 33% MON810 diet in the 90-day feeding trial E.

III) In the 1-year feeding trial no differences in the percentage of neutrophils between the control and the MON810-fed female rats were observed.

The Austrian colleagues point out to the increase in the percentage of monocytes in female rats in trial B. The following facts support the conclusion that the increase in the percentage of monocytes in the feeding trial B is not related to the feeding of the GMO-containing diets:

I) The percentage of monocytes was increased in female rats fed the diet containing the conventional maize variety 1 (the increase not being statistically significant) and was significantly increased in female rats fed the diet containing the conventional maize variety 2.

II) The percentage of monocytes remained unchanged in female rats fed the 11% MON810 and the 33% MON810 diet in the 90-day feeding trial D.

III) The percentage of monocytes was decreased to the same extent in female rats fed the 11% MON810 and the 33% MON810 diet in the 90-day feeding trial E, whereby the decrease became statistically significant in female rats fed the 33% MON810 diet.

IV) In the 1-year feeding trial no differences in the percentage of monocytes between the control and the MON810-fed female rats were observed.

The Austrian colleagues point out to the increase in the thymus weight in female rats in trial B. The following facts support the conclusion that the increase in the thymus weight in the feeding trial B is not related to the feeding of the GMO-containing diets:

I) The thymus weight remained unchanged in female rats fed the 11% and 33% MON810 diet in the 90-day feeding trials A, D and E.

II) In the 1-year feeding trial no differences in the thymus weight between the control and the MON810-fed female rats were observed.

3) In the 90-day feeding studies, blood samples could have been taken every week to enable further investigation of any significant findings in blood parameters which might indicate a detrimental effect.

This possibility, as well as other alternative test designs tailored to the specific demands of GM plant toxicity testing (i.e. restricted exposure levels of the animals due to the need to provide nutritionally balanced diets) are not discussed by GRACE.

Answer:

In the course of GRACE four 90-day feeding trials were performed. Studies A and B were performed by closely adhering to the standard procedures suggested by OECD and EFSA. Since increased sampling may stress the animals and may introduce further sources of variability into the trial, two separate pilot studies - D and E - with frequent sampling of blood and urine were performed and extended analyses of immunological and metabolomic endpoints followed. The data were presented and extensively discussed at the Stakeholder Workshop in October 2015 and were also summarized during the Final Conference in November 2015. The results are fully considered in the conclusions and recommendations by the GRACE project.

The project investigated a basic but limited set of test designs starting at the level of the current practice and by taking into account the request to perform 90-day feeding trials with whole GM food/feed on a mandatory basis (see ref. [7]), which implies that an appropriate test to detect potential subchronic toxicity of GMO-containing diets is available. The results of the GRACE project showed and confirmed that – if there is no trigger from the preceding analyses – 90-day feeding studies do not add relevant information to the risk assessment. Within GRACE, and in contrast to the statement by the Austrian colleagues, a strong focus was placed on the evaluation of alternative approaches that could be considered for the safety evaluation of GM whole food/feed. The trials performed in the frame of GRACE, the endpoints tested and alternative approaches investigated throughout the project cover a set of options that support further discussions. The project was well aware that not all potential modifications and combinations of designs and endpoints could be tested within the project. Regarding the limitations:

- the stakeholder consultations were undertaken to cover and document any critical reflection (these documents are publicly available at the project website);
- a platform for broad data sharing was implemented to enable further detailed analyses of the trial data (see http://www.cadima.info/);

- efforts were coordinated between the projects GRACE, G-TwYST and GMO90+ to provide a complementary data pool covering various trial options;
- a scientific discussion forum was established to encourage the detailed scientific examination of the GRACE findings and further elaboration of recommendations (see Archives of Toxicology 88: 2067-2069, 2014).

Therefore, the GRACE Conclusions and Recommendations do not suggest new standard procedures, but summarize the findings and indicate possible research options for further consideration. Granting access to detailed research data essentially supports further thorough discussions, and the GRACE team further promotes standard procedures for transparent data integration (systematic reviews and evidence maps).

4) In Chapter 5 (p. 31), the following question is raised by the GRACE Team: "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?" Such conditions are neither mentioned nor discussed. Thus, this question remains unanswered and important issues unclear.

Answer:

Chapter 5 places the recommendations provided in Chapter 4 (p. 29, 30) in the context of the triple R approach. The conditions mentioned in Chapter 4 are:

- 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.
- 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 expected magnitude of a distinctly identified potential effect is considerably higher than the detection limit of the methods used in the trial – it 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.

These conditions are key to consider which study design is appropriate to inform the risk assessment sufficiently. (see also: DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union L 276/33)

5) In the same Chapter 5, an omics approach is postulated to inform the development of a targeted hypothesis in the future in order to scientifically justify the performance of feeding trials.

But at the same time, it is declared, "standardized and validated test procedures are currently not available" for this new omics approach. (Chapter 2, p. 26).

Why does the GRACE Team present no discussion on potential alternatives as long as no validated omics approaches are available?

What is the assumption based on that a targeted hypothesis tailoring the study design to a posed safety concern can only be developed by an omics approach that is currently not validated? The GRACE Team provides no answer.

Answer:

The Austrian colleagues tend to interpret the GRACE recommendations as a "new standard" in GM crop safety testing. Essentially, it is not the intention of GRACE to claim new standard procedures based on the limited number of tests performed. GRACE intends to promote a broader scientific discussion about potential test systems and their added value in the context of the GMO risk assessment. Particularly, the GRACE team did not state "that a targeted hypothesis tailoring the study design to a posed safety concern can only be developed by an omics approach that is currently not validated."

At the same time omics analyses become common practice in plant breeding to identify even small differences in the physiology of plants of interest. In the scientific literature there are many papers that show that known differences between plant varieties can be confirmed by omics strategies.

The Austrian colleagues suggest a desktop study comparing potential alternatives. This was not the scope of this project work stream, nevertheless the publications to be provided by the GRACE team will partly review research activities linked to this topic. In addition, discussion on potential alternatives was / will be partly performed by another project work stream preparing evidence maps in the area of health impacts of GM crops that provide an initial overview as basis for future directed investigations.

6) According to a scientific paper published by the GRACE Project Team (2) "the statistical power was assumed to be 0.8 with a significance level of 0.05 and a two-sided test, and an SES of ±1.0 SD that would be achieved by 17 animals per group." It is further stated that "the number was rounded down to an even number (i.e. 16) as the rats were to be housed in pairs." At this stage it would have been possible to round up to the number 18 in order to enhance the power.

Answer:

The gain in statistical power when including 18 instead of 16 animals per group and sex is about 3%. As discussed extensively during the GRACE stakeholder workshops a further increase of the number of test animals is therefore not justified.

7) The statistical power of the test design could have been maximised up to 0.9, although in this case the inclusion of conventional groups in the feeding studies would not have been possible.

This argument is partially supported by Schmidt et al. (2015) (3) stating that significant differences must be evaluated with respect to their biological relevance and giving advice

that "this is equally true for non-significant differences as it would be unacceptable if biologically relevant effects went unnoticed for the lack of statistical power.

Schmidt et al. (2015) (3) further indicate that "For this reason, a prospective power analysis has been made mandatory in GMO risk assessment (EFSA Scientific Committee 2011)"(4).

Answer:

As required by EFSA a prospective power analysis has been performed and discussed with the stakeholders resulting in a study design to use 16 animals per sex in each treatment group of studies A and B. This design allowed us to include additional conventional varieties – as strongly recommended by several stakeholders – to generate historical data for the test facility and to gain more insight into the biological variability.

8) It is evident that statistical models and animal test designs used in GRACE are a compromise between different requirements and demands. In this respect, the general statement in Chapter 4 lacks a commentary exploring any alternative approaches.

Answer:

As already stated the GRACE project investigated a basic but limited set of test designs starting at the level of current practice and by taking into account the request to perform 90-day feeding trials with whole GM food/feed on a mandatory basis. There are further closely linked projects like G-TwYST and GMO90+ that explore complementary or extended designs. As described in Part II: Conclusions and recommendations on the use of systematic reviews and evidence maps when summarizing and evaluating GMO impact data (p. 39), evidence maps have been drafted on four health-related topics (toxicity of newly expressed proteins and whole foods/feeds, allergenicity, composition) showing that publications straddle a wide range of crops, newly introduced traits, experimental animal species and other experimental models and parameters employed.

The statistical analyses used in GRACE follow OECD recommendations and suggest advancements for data representations and analyses of time series data. The statistical models being used in GRACE were adequate to the experimental design and did not include further assumptions.

Only limited experimental data for alternative designs or modifications of animal studies with whole food/feed are currently available that allow a detailed analysis or demonstrate their added value. GRACE invited applicants and researchers in this field to openly share and discuss their experimental results to enable a critical overall examination. The invitation is still valid. In general, a substantial database including detailed recommendations for “standard approaches” is missing.

9) In Chapter 1.1. (p. 22), the GRACE Team notes that the "data gathered during the 1year feeding study (Study C) conducted in GRACE are said to concur with the conclusions made after 90 days that administration of maize MON810to rats had not shown adverse effects and had not provided relevant additional information compared with the 90day studies." This cannot be evaluated or commented, since raw data and statistical report of the 1-year feeding study have not been made available yet.

Answer:

As mentioned in the answer to Comment 2 of the Austrian colleagues, the complete data set (as means ± standard deviation of each endpoint) was available to Dr. W. Brüller and DI W. Stepanek, since they attended the GRACE Stakeholder Meeting in October 2015. Detailed data of the 1-year feeding trial have been provided to stakeholders who signed a Non-Disclosure Agreement in preparation of the stakeholder workshop in October 2015. This was required in order not to hinder any scientific publication by previous public distribution of the manuscript and the data. The Austrian experts attended the meeting and signed the agreement.

A similar procedure has been applied for providing access to full data before discussing the results of the 90-day studies A and B at a stakeholder consultation in Brussels in 2014. We want to highlight that access to data including raw data from the animal feeding studies for the purpose of facilitating stakeholder discussion is one of distinct attempts of the GRACE project to increase transparency beyond what is normally done. We are not aware of any other research group that made available the complete data set of a feeding trial before the manuscript was accepted for publication.

The manuscript for the 1-year study has been submitted to Archives of Toxicology. The detailed data will become publicly accessible with its open-access publication and provided on the website http://www.cadima.info/.

The GRACE team had requested at the European Commission a prolongation of the project for better temporal coordination of stakeholder involvement, collation of data and drafting of manuscripts. This prolongation was not granted for formal reasons. Therefore, the complete data will be published after the end of the project and considerably later than the stakeholder consultations and the final conference.

10) In Chapter 2 (p. 26f), it is said that a one-class model omics approach can provide a better basis for the decision on the scientific rationale to frame the subsequent risk assessment steps.

However, alternative settings (extending the numbers of endpoints in comparative assessments, enhanced molecular analyses, etc.) are not mentioned and seem to have not been evaluated by GRACE.

Combining different omics techniques could also be more promising, since changes at proteome or metabolome levels may not be detectable at transcriptome level.

Answer:

As stated in the Conclusions and Recommendations of the GRACE project, it is well feasible to make omics strategies part of a comparative compositional assessment. Omics data of the GM variety can directly be compared to the nearest comparator, preferably the near-isogenic control line. It should be noted that in the direct comparison on the basis of omics data and given the large number of end-points, when looking at individual end-points there will be many statistically significant differences (with the threshold value of p e.g. at ≤ 0.05)

in all cases. This is inherent to the approach when many end-points are involved. These observed differences can all be assessed separately, but this will be very labour-intensive.

To overcome this issue the statisticians from Wageningen UR together with the chemometricians from the University of Nijmegen, The Netherlands, have developed the one-class model: the model has been developed in a way that it will only classify new profiles as inside the one class of profiles that we consider as safe, when they show large resemblance to the profiles in the one class. Differences will relatively easily lead to a classification outside of the one class. The GRACE data have shown this: the GM maize varieties are classified inside the one class, profiles of experimental potato varieties that are more genetically distant from current commercial varieties (although safe for human consumption) are classified in most cases as outside of the one class, as are the fungi-infested maize samples not used for the feeding trials. But, all data being available, it is possible to perform the standard comparison (as is currently performed in targeted compositional analyses as part of all GMO risk assessments) based on the omics data as well. For the maize materials that were used in the two 90-day feeding trials A and B both the direct comparison as well as the comparison on the basis of the one-class model have been performed. The two comparisons showed the same outcome: the profile of the GM variety is very similar to the profile of the conventional counterpart, with no distinct differences that would require further risk assessment.

The maize materials have been assessed using transcriptome, proteome and metabolome strategies, the potato materials have been assessed using transcriptome and metabolome strategies.

11) With respect to the one-class model omics approach, it is referred to the identification of mycotoxin contamination in maize or the detection of Cry1Ab gene in MON810.

This raises the question whether this information is considered sufficient to provide answers if this model can detect any changes in plant transcriptome with potential toxicological relevance.

Answer:

This Comment of the Austrian colleagues seems to base on a misunderstanding. The mycotoxin-contaminated maize samples – not being included in the GRACE feeding trials – were included as test samples that were of inferior feeding quality. The samples were used to test whether the system would classify these samples as being inside or outside the one class on the basis of metabolomics profiles. It was assumed that the presence of the fungus would lead to physiological differences in the maize materials and this proved to be the case: the samples were classified as outside of the one class of profiles of maize varieties that we consider as safe.

The detection of the expressed Cry1Ab gene in MON810 was a separate assessment, based on the same transcriptome data, but has nothing to do with the use of the one-class model. The transcriptome data were assessed for the presence of newly expressed RNAs that were not known to the non-transgenic maize transcriptome. This assessment was performed in

two steps: In a first step, the transcriptome of the GM maize variety was compared to the maize genome. For the sequences that were not recognized in this way, a *de novo* synthesis was performed (build longer sequences based on similarity) and on the resulting sequences a BLAST analysis was performed (search very broadly to find the identity of these sequences). In this way, the Cry1Ab gene was identified.

The experiments show that these two different approaches have not failed in the cases to identify relevant differences or in the other cases that have been tested in the GRACE project (based on the potato materials).

12) The standard approach for GM plant comparative assessments focusses primarily on the identification of differences between a GM plant and its conventional counterpart, since this seems to be the most promising way to identify unintended effects caused by the genetic modification process.

The one-class model omics approach uses a different methodology with the aim to test if a GM plant belongs to a single class defined by conventional plant varieties based on its transcriptome profile (5).

In this regard, the one-class model omics approach clearly differs from the comparative assessment concept as defined by the Commission Implementing Regulation (EU) No 503/2013.

Answer:

See also the answer to Comment 10. Omics data can be used for a one-to-one comparison. The one-class model also aims to identify differences in the GM variety when compared to a set of commercial varieties of the same species that we consider as safe, but here the aim is to disregard small differences that do not lead to an intrinsic different profile, but focus on relevant differences that may need further assessment. At the same time, the model has been constructed in a conservative way: An aberrant profile will lead to classification outside of the one class; this has been demonstrated in the case of the experimental potato varieties, which are considered safe for human consumption, but are genetically more distant. In the majority of cases, the profiles of these varieties have been classified as outside of the one class, on the basis of the transcriptome as well of the metabolome data.

13) The information provided in the GRACE Final Conclusions and Recommendations remain limited and the provided discussion insufficient.

Details on important questions as e.g. alternative approaches or observed weaknesses due to data gaps or limited resources are not addressed. Pros and cons are not presented but more-or-less certain models (the SES approach, the one class model omics approach, etc.) postulated; in most cases without illustrating alternatives or differing concepts.

Answer:

The Conclusions and Recommendations are neither intended to present a detailed discussion nor setting new standards but to summarise the interpretation of the results being derived from the experiments performed by the GRACE consortium and

acknowledging the broad stakeholder involvement. Specific and detailed discussions are and will be provided within the scientific publications. GRACE also underlines that currently a detailed overview of research data on the added value of a range of methods is not available. Though several test systems have been employed in connection with rodent feeding trials with whole GM food/feed, an added value for the risk assessment has not been shown. Especially the lack of a positive control for whole food/feed is challenging. Therefore, GRACE urges a broader scientific discussion based on accessible data. The project implemented various options to promote this further including the broad stakeholder involvement and the detailed documentation of the stakeholder consultations on the GRACE web page.

14) At present, only the raw data on two 90-day feeding trials and two related publications have been made available. The CADIMA database also currently provides no reports or publications that present any results.

For a final broad discussion of the GRACE results and a continuing scientific debate that initiates the reappraisal of the requirement to mandatorily perform a 90-day feeding trial with whole GM food/feed (leading to a decision for the amendment of the Implementing Regulation (EU) No 503/2013), it is necessary that the scientific community and the involved stakeholders can study all raw data and results. This is not currently possible.

Answer:

See also the answer to Comment 13.

The data of the studies A and B are available at http://www.cadima.info/ by clicking in the field Animal Feeding Trials and accepting the open access licensing (CC BY). These data are available online since 2014. Further study data will become available the same way once the respective papers have been published.

15) The GRACE consortium fails to clearly communicate the limitations of the chosen approach concerning 90-day feeding studies with whole food and feed:

1. The evaluation was performed with MON810 varieties only (i.e. a first generation GMO with a single introduced transgene). The obtained results and conclusions are therefore only valid for the tested transgenic maize lines at best.

Answer:

A key question for the project was whether there is a (generic) added value of rodent 90-day feeding trials with whole GM food/feed. They should inform a clear decision on potential adverse effects caused by the GM food/feed beyond the information that is already available through other elements of the risk assessment. Such an added value could not be demonstrated – neither for the basic study design proposed by EFSA and realised for data gathering in the frame of applications nor for complementary methods. The limitations shown in the set of trials performed and methods used by GRACE led us to the conclusion and recommendation that an added value cannot be warranted in an untargeted approach and moreover may provoke misleading interpretations. Feeding trials with whole food/feed need guiding research questions and a distinct hypothesis on the likely effect to decide on an

appropriate study design that can inform the risk assessment. Consequently, this contradicts a mandatory 90-day feeding trial that follows a broader standard protocol.

2. No information about effects of GMOs with

a) stacked events or

b) introduced transgenes coding for different modes of action compared to MON810 or with

c) complex alterations of metabolic pathways (i.e. second generation GMOs, which are advancing rapidly to commercialization)

have been obtained during the 90-day feeding studies of the GRACE project. Generalization of the drawn conclusions on all possible GMO varieties is therefore scientifically questionable.

Answer:

The contractual research tasks of GRACE were not targeted to GM plants described in this Comment. There are further cooperating projects (G-TwYST and GMO90+) that will provide complementary information though stacked events and "novel" traits are also not covered by them.

The Comment made by the Austrian colleagues assumes that a (90-day) feeding trial has a definite added value in the cases listed above. Moreover, it implies that these GM plants will show more pronounced effects. If more pronounced effects are expected, then a targeted approach should be considered using an appropriate study design or other methods used in the risk assessment may provide adequate information for decision-making. Regarding the intrinsic limitations of 90-day feeding trials with whole food/feed, the Comment underlines the recommendations made by GRACE.

3. The minute amounts of the active principle (i.e. the Bt toxin, Cry1Ab) in the diet requires an absolute robust testing regime, highly skilled and trained staff and appropriate facilities for handling the necessary numbers of animals appropriately if differences between the verum and the control group are to be detected and are not to be concealed by laboratory-induced variation.

This requires

a) the involvement of laboratories with a renowned record of expertise in conducting 90-day feeding studies with whole food and feed in rats (the chosen animal model in the present project) and

b) experimenters at the animal testing facility with a longstanding expertise in the field of feeding studies with rats.

There are drawbacks regarding both requirements in the present project as laboratory-induced variation and strong circadian effects were conceded by the GRACE consortium. Additionally the laboratories and the study director responsible for performing the animal experimentation showed only little experience in that type of tests before involvement in the GRACE project.

Answer:

The Institute at the University in Bratislava essentially fulfils the criteria mentioned in the Comment made by the Austrian colleagues except for experience in trials performed with whole food/feed. It was a requirement in the project formulation and negotiation phases that the feeding trials should be performed in an institution not being involved before in commercial testing of whole GM food/feed to avoid any kind of conflicting interests. The institute routinely performs OECD feeding studies under GLP. The study director is highly qualified and has a long experience in planning, conducting and analysing of rodent feeding trials. Laboratory variations will occur in each laboratory. In the frame of a research project like GRACE that aims to test and improve study designs it is essential that potential shortcomings are considered and addressed thoroughly. The detailed documentation provided by GRACE allows reconsidering quality demands. In the context of the feeding trials with whole food/feed, the GRACE studies showed a random pattern of differences between test groups. The relevance of taking into account circadian rhythms when wanting to perform omics analyses underlines the need for a targeted adjustment of the study design when – in addition to a routine toxicological feeding trial – additional methods that track physiological dynamics are employed.

4. The active principle (i.e. Bt toxin Cry1Ab) was not detectable in some of the transgene containing diets applied as feed during the experiments.

Answer:

The statement by the Austrian colleagues is incorrect. The active principle i.e. the Cry1Ab protein and the PCR product of the MON810 event have been detected in all diets containing the transgenic varieties. In contrast, the cry1Ab gene could not been amplified by PCR for some diets. The reason why the PCR failed is unclear (e.g. quality of primers).

5. Some of the GM-free diets were contaminated to some extent with the Bt toxin. This observation is of importance because control groups receiving Bt-contaminated feed might further blur active principle induced alterations between verum and control.

Answer:

The statement by the Austrian colleagues is not precise. The Cry1Ab protein was detected in the conventional non-transgenic maize varieties. In the near-isogenic varieties (controls) the level was less than 1% of the level in the transgenic maize varieties. Cry1Ab was below the level of detection in the diets containing the near-isogenic controls but present in two out of four diets with conventional maize varieties. Traces of the event MON810 (detected but not quantified) has been detected in the control diet of trial A, but has not been detected in the control diet of trial B. Therefore, the content between the transgenic diets and the control differ by more than 2 orders of magnitude. It can be concluded that the trace amounts of Cry1Ab protein definitely did not influence the outcome of the feeding trials.

6. Stakeholder consultations have been regarded as a core element of the GRACE project, covered even by a dedicated work package. The opportunity to engage in an open-minded dialogue between the GRACE consortium and several stakeholders is mirrored in the

"Conclusions and recommendations" of the GRACE consortium and certainly welcomed. But it is astonishing that none of the concerns expressed by the stakeholders have been picked up and discussed in this final report. Communicating the status of the discussion on disputed aspects of the project should have been a primary task of this project to provide an unbiased representation of the issue. GRACE missed this opportunity.

Answer:

The discussions during the stakeholder consultations and the written stakeholder comments have been reported in detail. The reports are published on the GRACE website. Due to the broad stakeholder representation and involvement it is obvious that plenty of diverging opinions have been presented and documented. The GRACE Conclusions and Recommendations are based on extensive internal discussions taking into account all stakeholder comments as reflected by the written responses of the GRACE team included in the stakeholder consultation reports. They do not claim to represent particular stakeholder opinions or statements and do not intended to preferentially highlight some of the stakeholder statements.

7. The GRACE consortium is of the opinion that feeding studies with whole GM food/feed „might be considered, …if safety concerns are raised during the molecular, compositional, phenotypic and agronomic analyses of a GM plant."

We would like to point out that in the given cases feeding studies with whole food/feed are already requested at present by the relevant EFSA guideline (6) and the regulative GMO risk assessment framework (7-9) currently in force. Under the respective conditions performing a whole food/feed study is mandatory and not only a suggested possibility.

Answer:

The statement of the Austrian colleagues is correct. Feeding trials are a mandatory legal requirement in the EU. However, as indicated in the Commission Implementing Regulation 503/2013 the EC will review this provision and will consider (among other) the results of GRACE. The Implementing Regulation states that "The current uncertainties in relation to the need and design of 90-day feeding trials will be addressed by a large research project under [...] the seventh Framework Programme for Research (FP7). The requirements regarding animal feeding trials in the context of GMO risk assessments should be reviewed in the light of the outcome of this project expected to be available by the end of 2015 at the latest."

Furthermore, if safety concerns are raised by other risk assessment elements and a targeted hypothesis can be derived from the gathered results, the approach chosen to test the hypothesis should be targeted to the posed safety concern. Consequently feeding trials with whole food/feed may not necessarily represent the most appropriate test design and alternative approaches should be considered as well.

8. We would like to stress that 90-day whole food/feed studies (as envisioned by EFSA)(4) are low-sensitivity test settings (as acknowledged also by the GRACE consortium).

9. We would like to underline the fact that in the course of the GRACE project MON810 showed significant differences between verum and control groups (even in this low-sensitivity setting of a 90-day feeding trial with transgenic plants). Additionally, clear trends of effects – albeit not surpassing the statistically set significance levels - induced by MON810 in the verum group are documented in the study results. These trends could be also observed versus conventional varieties-fed groups, thereby strengthening the evidence. GRACE - conceptualized as research project - would have had the obligation to investigate the cause and nature of these aberrations but missed this opportunity.

Answer:

For details see answer on Comment 2. Interestingly the Austrian colleagues suggest considering the additional conventional varieties though they state in their Comment 1 "the comparison against a historical control (or conventional groups included in the test system) weakens the statistical significance because of increasing the statistical variance." These additional control data are now used as an argument that "these trends could be also observed versus conventional varieties-fed groups, thereby strengthening the evidence" while initially it was suggested not to include such data. Hence, it is now acknowledged by the Austrian colleagues that the use of historical controls provides a considerable value for the interpretation. As already described in the answer on Comment 2, the analyses of the differences and other observations did not indicate adverse effects. The further GRACE studies (Schmidt et al. [2016], manuscript in preparation) also show that the observable differences vary between studies and are not specifically related to the genetic event. This finally led to the conclusion that 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.

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