Reply to letter to the editor

First of all we thank (Korsaeth et al. 2014) for the opportunity to discuss some important topics: comparative methodology, quality testing and risk assessment of plant material for food and feed. In the article under discussion (Bøhn et al. 2014), we used a comparative approach, which goes beyond compositional assessment of crops from strictly controlled test-plots. Contrary to comparative assessments following established guidelines requiring near-isogenic plants, we attempted to compare plant products representative of different agroecological systems. We acknowledge that this methodology of comparing ”market-ready agricultural plant products” instead of ”products from test-plots” has its specific challenges, and we acknowledge the points made by Korsaeth et al. However, we wish to use the opportunity to detail the discussion of several important issues related to risk assessment and testing of plant products, including strengths and weaknesses of different comparators in the testing of genetically modified (GM) plants. Hopefully this will inspire further research.

Research evolves through scientific discourse and may be improved by critique as well as by evaluation of the criticism, and by putting the research and its limitations in perspective.

We are happy to see that the key result demonstrating high residue levels of glyphosate and AMPA in GM soybeans is appreciated. The average residue level from n = 10 samples of Roundup-Ready GM soybeans was 9.0 mg/kg glyphosate. Herbicide residues have not been regularly monitored and have never been part of the GM crop risk assessment process anywhere for the nearly 20 years during which Roundup Ready soy and hundreds of other herbicide tolerant GM crop events have been commercialized. This is quite a remarkable fact. Residues of pesticides at the mg/kg (= ppm) level should not be forgotten for what they are, namely, an important and health-relevant “compositional element” of the plant or food product.

Korsaeth et al. claim to see “*fundamental errors of methodology*” in our paper, particularly the sections that focus on analyses of nutrient and element concentrations in soybeans. The central issue that Korsaeth et al. raise is that certain factors confound our analyses of the levels of specific nutrients and elements, making it unjustifiable to compare directly the three categories of soybeans, included in our study. The factors that Korsaeth et al. are concerned about were: (I) soybean variety (genetics of the soybeans), (II) yield level, (III) soil properties, and (IV) fertilizer use. In addition they criticize the ranking of organic soybeans as healthier than conventional and GM soybeans (V). We are well aware of the fact that factors I-IV influence soybean composition, but uphold the value of such broad comparisons as we do in the paper. We respond first specifically point by point and later with some more general points on comparative risk assessment.

Specific points:

Factors influencing nutrient/elemental composition

I. **Interactions between nutrient/element concentration and soy variety**
   Korsaeth et al. seem to misunderstand a couple of our messages in the paper.

First, they claim that we use a "*single 'variety-overlap' [the one with Legend 2375] as evidence of there being little impact of 'genetic background' on varietal differences in soybean composition*". Secondly, Korsaeth et al. "*fail to see how it was possible to draw any conclusions on genotype x environment interactions*." (i.e. based on our data set).

To the first point: what we emphasized in the paper was that "*variation in composition will come from all three of these sources*" (i.e. from variety/genetics, environment and agricultural practice), and that our samples included "*representative data regarding soy composition from that particular region. To test food products that are not experimentally matched, e.g., for different soil conditions, resembles the situation for a consumer in the store.*" (Bøhn et al. 2014, page 211 bottom, left).

To the second point: a study that should test and conclude on specific genotype x environmental interactions would clearly need to have a factorial design with a range of experimental treatment groups, varying both genetic background and environmental conditions, not simply buying soy on the market, as we did. Thus we only 'observed' the presence of likely genotype x environment interactions by noting that pairs of the same variety could both be similar and different in composition.

We cannot exclude that soy variety performance may have accounted for some differences between the agricultural practices we tested, but we find it unlikely to have been a key factor in determining composition in our data set. Some support for this claim come from the fact that the 'pairs' of the same varieties rather spread out than matched in the cluster analysis, i.e. in composition (fig. 2b).

In general, two classes of soybean are planted in Iowa. The primary category, accounting for, by far, the largest acreage consists of varieties used for animal feed and commodity food and industrial applications. The second category, specialized "food-grade" varieties are used for applications such as tofu production. The most important quality/nutrition parameter for both categories is protein content. Beans in the latter category are consistently of higher protein concentration. However, within a given category protein content is relatively consistent, as is oil content. For our study, only beans in the former category—commodity beans—were used.

Although variety genetics might for example have contributed differences in protein content of a fraction of a percent, we find it unlikely that genetics explains a difference of two percent, as seen between the organic and the two industrial soybean types.

An alternative design for our study could have been to compare ten pairs of isogenic lines, wherein each pair differed only in the presence of one or more transgenes. The variety in each pair that lacked the transgene(s) would have been grown according to both conventional and organic practices. However, because it is virtually impossible to obtain such test materials for research from the companies that create and commercialize GM crop plants (c.f. Nielsen 2013; Sissener et al. 2009; Waltz 2009b; Waltz 2009a), such alternative approaches to compositional studies are little more than theoretical possibilities.

II. Interactions between nutrient/element concentration and yield

We acknowledge this point from Korsaeth et al. We cannot exclude that yield differences may have contributed differences in nutrient/element differences between organic and industrial (conventional and GM soy). However, it is not likely to have been a major confounder in our data set. First, differences between farmers in yield from the area of sampling are unlikely to have been large, because professional farmers, such as those from whom samples were purchased, fine-tune production practices to optimize their operations. The parameters that can be fruitfully optimized will be quite similar for all farmers in this region, where soil quality (see below), weather (water availability) and other environmental factors are quite similar, and where the products sampled are produced using soybean varieties that have similar basic agronomic properties (except for Roundup tolerance). Because of similarities of soil, genetics and environment, and because farmers in the area basically are optimizing their practices based on the same target—maximal yield—we would expect farmers to converge on similar fertilizer application rates throughout the region, regardless of the production system used, GM, conventional or organic. Thus, roughly the same agronomic performance and yield can be expected in samples that come from well managed farms, such as our soybean samples.

The discussion on yield is both important and complex: In a ”perfect growing season”, when rain and temperatures are optimal and without major pest problems, the industrial/chemical production typically perform somewhat better than organic. But in years where there is drought or too much rain or when other factors are suboptimal, this may be reversed. Organic agriculture aims more at long term sustainability and avoidance of non-renewable inputs, likely at some cost for the average yield.

III. Interactions between nutrient/element concentration and soil properties

Soil properties were not highly variable. There are seven major land forms in Iowa, each of which is associated with a unique principle soil type association. All of the soybeans used in our study were produced in the same land form, specifically, the Southern Iowa Drift Plain landform, which is characterized by Loess-derived soils. There are only two primary classes of these soils present in this area of Iowa, those formed under prairie and those formed under forests. Both of these soil types are known to be highly suitable for soy cultivation.

IV. Interactions between nutrient/element concentration and fertilizer use

The rate of fertilizer applications, as mentioned above, is in our case likely to be similar between the different types of production systems. However, the types and quality of fertilizers differ between organic and industrial farmers. This may contribute to differences in availability of nutrients in the soil, and thus in plant uptake, as detailed by Korsaeth et al.

V. **Ranking agricultural practices in terms of a healthy nutritional profile**

We acknowledge the specific critique by Korsaeth et al. regarding our statement in the article that organic soybeans showed the healthiest nutritional profile. Admittedly, this statement should have been specifically and consistently substantiated by the nutritional data to be justified in the abstract of the paper. However, our data set demonstrate that GM, conventional and organic soybeans are systematically and consistently different in their composition. This is in itself a significant result. In particular, it brings into question the validity of the safety assessments based on 'substantial equivalence' and feed into the discussion on what are the most relevant comparators.

Furthermore, we have performed supplementary studies with the same soy samples, carrying out extensive feeding experiments in *Daphnia magna*, to assess more deeply the nutritional quality of the three soy types of interest. Based on 18 different soy treatments, including low versus high dose, raw versus heat-treated soy, low versus high inclusion rate of soy, we have demonstrated that animals fed organic soybean show better growth, higher reproduction rates and lower mortality (Cuhra et al. 2014, in press). In particular, we found large differences in fitness of the test animals feeding on *raw soy*, i.e. when there was direct match to the nutritional measurements under discussion. Heat treatment of the soy used as feed leveled out some of the differences in the feeding studies (Cuhra et al. 2014, in press). These results support that organic soybean have a higher overall quality as food/feed as compared to transgenic and conventional varieties. Both glyphosate residues and the nutritional profile of the different categories of soy may explain these results since i) the conventional soybeans (that did not contain any glyphosate residues) performed less well than the organic, and that ii) GM soybeans (that contained glyphosate residues) performed inferior to both other types of soybean.

We have also tested the significance of the *amount* of glyphosate residues in the GM soy, by feeding *D. magna* diets from different GM soy samples, in which glyphosate residues varied by a factor of more than 10 (Cuhra et al. in prep.). The curious reader will have to wait for the interesting results of that feeding trial until it is properly published.

General points:

Limits in all comparative risk assessment studies

All research activity is exerted within limitations. We suggest that Korsaeth et al. should view the perceived shortcomings and limitations in our study in the context of the limitations of other relevant comparative-assessment studies (e.g Worthington 2001; Woese et al. 1997). Special emphasis should be put on the studies submitted to regulators as part of the safety assessments of GM products such as glyphosate-tolerant crops.

Unique data for comparing soy from different agricultural systems

We provide in depth compositional data, including residues of pesticides such as organochlorinated pesticides, glyphosate and AMPA and we have analyzed more than 90 nutritional components, comparing three different production systems of soy, with n = 10-11 samples for each of these agricultural systems. Such data from representative field conditions has, to our knowledge, never before been published, and is therefore a valuable contribution to the literature. Data on pesticides including glyphosate residues in glyphosate resistant plants should be regularly monitored, which would be a very useful contribution to the dossier submitted to regulators as part of the regulatory approval process for such GMOs.

As described in the *Food Chemistry* article, we tested the average quality of "ready to market" soybeans. That is, we sampled the systems in which soybeans are produced for the commodities market. On the market, i.e. for any consumer, information on the yield, the specific soil quality, etc. is not available. This is a limitation in our study. However, our data set included information on the background genetics (variety), field location, pesticide use and pesticide residues, in addition to the nutritional components. We therefore argue that this dataset serves as the basis for a valuable, market-relevant broad comparison of product quality. It can always be said that it would be better to have more data, and we agree with Korsaeth et al. that data on yield, fertilizer composition, doses and timing, and soil quality would be interesting and valuable. However, no single study can be expected to include all such possible confounding factors. Furthermore, as discussed below, experimental design in line with Korsaeth's comments and in line with established guidelines (EFSA 2011) is not possible today due to restricted accessibility to research materials, in particular for GM plant material protected by intellectual property rights (Nielsen 2013).

Specific, general and outdated comparators

It is interesting to note that regulations for assessing biosafety in Europe require the comparison of the GM variety or event with an isogenic or near isogenic line. In some cases, such comparisons reveal significant compositional or functional differences between the GMO and its isogenic comparator (Zolla et al. 2008; Jiao et al. 2010)

Such differences should trigger more in-depth safety assessment of the GMO in question. However, the developer of the GMO often presents data from a wider range of varieties of that crop, arguing that although the GM plant is significantly different from the isogenic line, it falls within the range of composition and functionality reported for the other, wider range of varieties of the crop of interest.

For this comparison, the applicant company often uses data obtained, not only from crop material cultivated in parallel with, and under comparable conditions to, the GM plant, but also obtained from material grown under a wide and highly divergent diversity of conditions. An example of this is the paper by (Hammond et al. 2006), claiming to demonstrate that a GM maize variety, MON810 is substantially equivalent to conventional maize. Several "reference" maize varieties were included in the comparisons (also feeding studies in rats), along with the GM variety and the unmodified isogenic maize line. Re-analysis of the data from the rat feeding studies showed that, for certain blood parameters, the GM variety was significantly different from the isogenic line, but significance was lost when the "reference" comparator lines were included in the calculations (de Vendomois et al. 2009). In many cases, applicant companies also use what is termed "historical control data" for comparison. Historical control data is obtained by combing the literature for data on composition and/or function of that crop. Historical control data will be obtained from papers often published 20 or 30 years earlier. The International Life Sciences Institute (ILSI), an industry lobbying group, has created a database of historical control data that is routinely used by industry in its applications for commercialization of GMOs (http://www.ilsi.org/FoodBioTech/Pages/CropCompositionDatabase.aspx). The historical data sets contain values generated under strikingly different (often outmoded and outdated) analytical methods and strikingly different cultivation conditions. Interestingly, as discussed by (Antoniou et al. 2012), there are many examples in which such data has been accepted by regulators as suitable for comparison to a GM crop variety, and used to justify acceptance of applicant companys' claims that the GM crop is "substantially equivalent" to the native crop.

In light of these practices, some of the deficiencies that Korsaeth et al. see in our paper should be put in perspective. Certainly, Korsaeth et al.'s characterization of "*fundamental errors of methodology*" would be applicable to some of the safety assessment procedures that are currently applied to GM crops in Europe and many other countries.

Herbicide residues overlooked in feeding studies

When it comes to feeding studies the present situation shows fundamental flaws in the testing procedures. Even though herbicide tolerant GM crop plants always will be sprayed by the farmer, most feeding studies are not using sprayed test material. Reviewing all of the published feeding studies for herbicide tolerant GM plants, in broilers, mice and rats (n = 16 studies), only three of them were found to have used sprayed plants (Viljoen 2013). Those 13 studies using unsprayed herbicide tolerant GM plants may be of some value, but they fail to assess the safety and quality of the HT crops as they are found on the market. Specifically, they fail to assess any potential effects of herbicide residues alone or in conjunction with the GM crop.

Regulatory policy for GM crops

Two points relevant to regulatory policy for GM crops emerge from our research and from the present discussion of that research.

First, the current safety assessment practices in Europe and many countries suffer from "*fundamental errors of methodology*," to use the terminology of Korsaeth et al. Specifically, although the applicant GM crop is compared to an isogenic line as part of the assessment, the decision as to whether more in-depth assessment is needed is not based on that comparison but on comparison with a much wider dataset (including data from "reference" varieties and "historical" data) that profoundly lack relevance. At present, GM seed industry is putting significant pressure on regulators to no longer require comparison between the applicant GM crop and the corresponding isogenic line. Regulators at EFSA have responded to this pressure by publishing a position paper supporting the use of a wider range of comparators (EFSA 2011) http://www.efsa.europa.eu/en/efsajournal/doc/2149.pdf).

We strongly recommend that the requirement for comparison with an isogenic line remain in place, that the legally mandated requirement finally be put in force, and that more in-depth safety assessment be conducted if significant compositional or functional differences between the GM crop and its isogenic comparator are observed. The current practice of allowing applicants to avoid more in-depth assessment based on comparisons with other, non-isogenic crop lines of diverse genetics produced under a diversity of cultivation practices should not be allowed to continue, since it is actually not consistent with the law.

The second policy point which emerges is that companies applying for commercial release of GM crops into the environment should no longer be allowed to create barriers that block independent researchers from access to GM crops for research purposes, and they should be required to make available relevant test material, including the isogenic line relevant to each GM crop commercialized. The lack of access to these research materials for independent researchers creates conditions wherein there is no independent oversight of the safety assessments provided by companies when applying for commercialization of GM crops. This is not in the best interests of the public.

Conclusion

We have responded to the specific criticisms by Korsaeth et al. by detailing the information on soybean variety (genetics), yield, soil type and fertilizer use for the analyses of soy composition in our original paper. We acknowledge some relevant and good points of criticism from Korsaeth et al., but we also argue for the value of broader comparisons of crop products produced under different agroecological systems, like GM, conventional and organic agriculture. Further, the restrictions of research material from GM crops strongly limit independent research. This is not acceptable.

We also provide a perspective on the criticism by highlighting serious flaws in current test practices for GM plants. In spite of some limitations in our study design, we are still convinced that our paper is important as it raises relevant questions on the safety evaluation of GM crops. This is particularly true with reference to residues of herbicides in herbicide tolerant GM plants; these residues simply fall outside the remit of regulatory authorities. Thus herbicide residues are insufficiently monitored, they not part of the regular risk assessment procedure, and they are not included in most feeding studies. But you will find them in Roundup Ready GM soy products on the market.

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References

Antoniou, M., Robinson, C. and Fagan, J. 2012. Teratogenic effects of glyphosate-based herbicides: Divergence of regulatory decisions from scientific evidence. - Journal of Environmental Analytical Toxicology S4:006.

Bøhn, T., Cuhra, M., Traavik, T., Sanden, M., Fagan, J. and Primicerio, R. 2014. Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. - Food Chemistry 153: 207-215.

Cuhra, M., Traavik, T. and Bøhn, T. 2014. Life cycle fitness differences in *Daphnia magna* fed Roundup-Ready soybean or conventional soybean or organic soybean. - Aquaculture Nutrition (in press).

de Vendomois, J. S., Roullier, F., Cellier, D. and Seralini, G. E. 2009. A Comparison of the Effects of Three Gm Corn on Mammalian Health. - International Journal of Biological Sciences 5: 706-721.

EFSA 2011. Guidance document on selection of comparators for the risk assessment of GM plants. - EFSA Journal 9: 1-20.

EFSA 2011. Guidance for risk assessment of food and feed from genetically modified plants. - EFSA Journal 9: 2150.

Hammond, B. G., Dudek, R., Lemen, J. K. and Nemeth, M. A. 2006. Results of a 90-day safety assurance study with rats fed grain from corn borer-protected corn. - Food and Chemical Toxicology 44: 1092-1099.

Jiao, Z., Si, X. x., Li, G. k., Zhang, Z. m. and Xu, X. p. 2010. Unintended compositional changes in transgenic rice seeds (Oryza sativa L.) studied by spectral and chromatographic analysis coupled with chemometrics methods. - Journal of Agricultural and Food Chemistry 58: 1746-1754.

Korsaeth, A., Riley, H. C. F. and Bakken, A. K. 2014. Comments on the recently published study: "Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans", by T. Bøhn, M. Cuhra, T. Traavik, M. Sanden, J. Fagan and R. Primicerio (Food chemistry 2014, 153: 207-215). - Food Chemistry (Letter to the editor).

Nielsen, K. M. 2013. Biosafety data as confidential business information. - PLOS Biology 11: 1-6 (doi:10.1371/journal.pbio.1001499).

Sissener, N. H., Sanden, M., Bakke, A. M., Krogdahl, A. and Hemre, G. I. 2009. A long term trial with Atlantic salmon (Salmo salar L.) fed genetically modified soy; focusing general health and performance before, during and after the parr-smolt transformation. - Aquaculture 294: 108-117.

Viljoen, C. 2013. Letter to the editor. - Food and Chemical Toxicology 59: 809-810.

Waltz, E. 2009a. Battlefield. - Nature 461: 27-32.

Waltz, E. 2009b. Under wraps. - Nature Biotechnology 27: 880-882.

Woese, K., Lange, D., Boess, C. and Bogl, K. W. 1997. A comparison of organically and conventionally grown foods - Results of a review of the relevant literature. - Journal of the Science of Food and Agriculture 74: 281-293.

Worthington, V. 2001. Nutritional quality of organic versus conventional fruits, vegetables, and grains. - Journal of Alternative and Complementary Medicine 7: 173.

Zolla, L., Rinalducci, S., Antonioli, P. and Righetti, P. G. 2008. Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. - Journal of Proteome Research 7: 1850-1861.