New EU Legislation for Risk Assessment of GM Food: No Scientific Justification for Mandatory Animal Feeding Trials

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Summary
This commentary focuses on the potential added value of and need for (sub)-chronic testing of whole genetically modified (GM) foods in rodents to assess their safety. Such routine testing should not be required since, due to apparent weaknesses in the approach, it does not add to current risk assessment of GM foods. Moreover, the demand for routine testing using animals is in conflict with the European Union (EU) Commission’s efforts to reduce animal experimentation. Regulating agencies in the EU are invited to respect the sound scientific principles applied to the risk assessment of foods derived from GM plants and not to interfere in the risk assessment by introducing extra requirements based on pseudo-scientific or political considerations.

Introduction
There is an intense discussion ongoing amongst scientists, regulators, NGO’s and politicians on the need for (sub)-chronic testing of foods/feed derived from genetically modified (GM) plants. This debate has, in particular, been fuelled by a recently published long-term animal feeding study that reports the toxicity of a herbicide-tolerant GM maize NK603 (Séralini et al., 2012). This 2-year study in rats appeared to reveal tumours in male and female animals, together with adverse pathological effects and biochemical alterations in liver and kidneys of treated animals. The authors suggested that ‘agricultural edible GMOs and formulated pesticides must be evaluated very carefully by long-term studies to measure their potential toxic effects’.

Recently, the EU Standing Committee on the Food Chain and Animal Health (SCoFCAH) adopted a Commission Implementing Regulation on applications for authorization of GM food and feed in the European Union (European Commission, 2013). The Regulation follows, for the greater part, guidance developed by the European Food Safety Authority (EFSA) for the safety assessment of the foods/feed derived from GM plants (EFSA, 2011a). However, unlike EFSA guidance, the new regulation demands a 90-day feeding trial in rodents for every single transformation event and, in specific cases, the same trial for plants containing transformation events stacked by conventional crossing. EFSA guidance recommends this type of experimentation only under certain conditions.

Current practices of safety evaluation of foods
Traditional foods are normally not subjected to systematic toxicological and nutritional testing because these foods have, in most cases, a long history of use and consumption by humans/animals and have not shown adverse effects. There are, however, exceptions where (acute) toxic effects have been observed in humans, for example, with raw soya flour, potatoes containing high levels of glycoalkaloids and cassava containing cyanogenic glycosides. Such compounds, which can include antinutrients, toxicants, bioactives and allergens, are assessed on a case-by-case basis.

Whole foods and food ingredients that have not been used for human consumption within the EU prior to 15 May 1997 fall within the scope of the Regulation (EC) No 258/97 (EC, 1997). The safety assessment of novel foods occurs on a case-by-case basis and requires data on the source, composition, expected use and exposure, previous human exposure and potential toxicity and allergenicity. Single substances or simple mixtures are tested like food additives following specific guidelines with the aim of establishing an acceptable daily intake (ADI). Whole food testing is not normally needed. So far, no new plant varieties per se have actually been assessed under the Novel Foods Regulation.
Difficulties with toxicological/nutritional testing of whole foods

The difficulties associated with the safety and nutritional testing of whole foods are well known. Whole foods contain thousands of different bioactive substances (macro- and micronutrients, antioxidants, antinutrients and natural toxins), which cannot be tested as individual substances. Furthermore, the quantities of food that can actually be incorporated into the diet of test animals will be limited and may not be high enough to induce adverse effects and to assess the potential toxic profile of the test food. These limitations are linked to the bulkiness of the food and the nutritional imbalances that addition of the test food may create. There is general consensus amongst toxicologists that animal feeding trials with whole (GM) foods are difficult to perform, have a low power to detect adverse effects, carry inherent risks of matrix effects and contribute little, if anything, to the safety evaluation of whole foods. Therefore, this type of study should, in principle, be avoided and far better analytical, molecular and toxicological methods should be applied for the risk assessment of GM foods. Such methods will have a proven capacity to identify unintended effects resulting from plant breeding, including genetic modification.

Risk assessment approach for the safety evaluation of GM foods

The placing on the market of GM food crops has resulted, at both national and international level, in the development of approaches for the safety and nutritional assessment of these products (EFSA, 2011a; FAO/WHO, 2004). There is general agreement amongst producers, food safety experts, risk assessors and regulatory authorities that the guidelines for risk assessment of these crops are robust and guarantee a high level of safety of these products once a positive evaluation has been completed.

The risk assessment strategy for GM plants and derived food and feed is focused on the comparison between GM plants and derived food and feed and their respective comparators. The underlying assumption of this comparative approach is that traditionally cultivated crops have gained a history of safe use for consumers and/or domesticated animals. For toxicological testing to be robust and efficacious, it must be proportionate to the potential source(s) of new hazards, which, for GM products, may arise inter alia from the transformation process, from newly expressed substance(s) or via some perturbation of the plants composition. To address these hazards, the principal components of the risk assessment are (i) the molecular characterization, (ii) comparative agronomic, phenotypic and compositional analysis of the GM plant and derived food/feed and its traditional counterpart, (iii) toxicological assessment of newly expressed compounds and of constituent compounds the levels of which may have changed as a result of the genetic modification, (iv) the assessment of potential allergenicity of the novel protein(s) as well as of the whole food derived from the GM plant and (v) the nutritional assessment of the food/feed derived from a GM plant. Specific toxicity tests such as repeated dose-toxicity studies using laboratory test animals may be needed for the characterization of single substances (e.g. newly expressed compounds including proteins) and to assess the consequences of any compositional alterations (e.g. in the content of micro- and macronutrients, natural toxins/antinutrients (EFSA,2011a).

Given the limitations of animal feeding trials with whole GM food/feed, these experiments should only be performed where necessary and EFSA (2011a) has indicated examples of conditions where such studies may be useful. These include (i) when the genetic modification has resulted in multiple and complex compositional alterations, (ii) when there is uncertainty and safety concerns on the possible occurrence of unintended (off target) effects (e.g. where new open reading frames (ORFs) are produced with similarities to toxins/allergens, or where metabolic pathways of the plant have been perturbed) or (iii) when there are indications for interactions between single events that have been stacked. This list of conditions is not exhaustive, and decisions should be made on a case-by-case basis, taking into account developments in our understanding of the potential impacts of genomic perturbations.

Where deemed necessary the objectives of the 90-day studies should be clearly indicated, that is, is the study confirmatory or exploratory? What are the biological endpoints to be investigated? What is the capacity of the study to identify certain adverse effects (experimental power)? EFSA has recently issued specific guidance on how to perform animal feeding trials (EFSA, 2011b).
The predictive capacity of subchronic animal tests to detect adverse effects of a toxic substance present in a food has been examined (EFSA, 2008 and references therein). It was estimated that the concentration of a toxic substance produced as a consequence of the genetic modification, in GM maize for example, needs to be at least 4000 mg/kg in the animal diet in order to induce adverse effects, assuming a median toxic potency. Substances present in smaller amounts or with a lower toxic potency will therefore not be detected in this type of feeding experiment, whereas most analytical detection methods have a much greater sensitivity with respect to detection and quantification. Thus, the capacity of a 90-day rodent test to evaluate the safety of whole food/feed is relatively low.

The optimal duration of rodent tests (subchronic or lifespan) to detect adverse effects has also been addressed (EFSA, 2008 and references therein). Extensive experience has been gained with short and long-term safety and nutritional testing of irradiated foods, and such studies have provided no indication of toxicological risks. Furthermore, no benefits of long-term testing were observed. Studies with different and specific substances (pesticides, pharmaceuticals, contaminants) in 3-month subchronic and 24-month studies in rodents indicated that in a majority of cases, the appearance of adverse (non-tumour) effects seen in the 2-year tests were also observed in the 3-month studies. In some studies, new findings were observed in the 2-year tests. However, these studies are not strictly comparable because they were carried out in different laboratories and at different times. Thus, the testing of chemicals in a 90-day trial with rodents is generally of sufficient duration to detect adverse effects. Whilst there may be cases where further chronic testing is justifiable, this should be decided on a case-by-case basis depending on the type and characteristics and available knowledge of the substance(s) under scrutiny. It is noted that the chemicals tested are far more diverse in their toxicological or pharmacological profile than those from GM plants where DNA, generally recognized as safe (GRAS) and target protein(s), is intentionally selected based on their biological function and history of safe use.

(Sub)-chronic animal studies with GM foods
Numerous animal feeding trials with laboratory test animals (rodents) and target animals of short (14–28 days) and prolonged (90 days to 6 months to 2 years) duration have been carried out. Multigeneration studies (2–5 generations) have also been performed. The test materials used include whole food/feed derived from GM maize, potatoes, rice, soybeans, tomatoes, rapeseed, triticale etc. (see for review EFSA, 2008 (Table 2), Snell et al., 2012; Ricroch, 2013). Standard parameters have been investigated including body weight, feed consumption, blood and clinical chemistry, organ weights and (histo)pathology. To date, no animal feeding trials (both short term and long term) performed according to generally agreed quality standards with whole foods/feed derived from GM plants have been able to identify any adverse effects. It is therefore unlikely that animal feeding trials can provide any extra information in the case of whole GM food/feed. Furthermore, in the light of current discussions on chronic testing of whole food/feed derived from GM plants, it should be emphasized that there is no scientific rationale for these types of study.

In some published studies, differences have been observed in test parameters measured in animals exposed to GM test material or exposed to control material, but many of these differences could not be interpreted or associated with the GM test material, due to deficiencies in the experimental design. Deficiencies include the lack of information on the source and production of the GM test material, lack of appropriate controls, lack of information on the composition of the administered diets, the use of diets that are potentially unbalanced nutritionally, lack of dose response or insufficient or no information on natural variations in test parameters. This information is needed to place differences potentially observed between the GM test material and its control into the appropriate biological context.

A recent example of poor experimental design is the long-term toxicity study of a herbicide-tolerant GM maize NK603, reported by Séralini et al. (2012). Various agencies responsible for food safety in EU countries and outside of the EU have criticized the experimental design used and the claims made by the authors that NK603 maize cultivated both with and without the use of Roundup herbicide, the active ingredient glyphosate, caused adverse effects in rats. The effects included development of mammary tumours in females and kidney and skin tumours in males. These agencies that criticized the study include EFSA (EFSA, 2012), the Australian and New Zealand Agency ‘Food Standards Australia and New Zealand’
(www.foodstandards.gov.au), the Health Canada and Canadian Food Inspection Agency (www.hc-sc.gc.ca) and the French National Academies of Sciences (www.academie-sciences.fr). The key limitations of the study include (i) the use of a rat strain with a well-known spontaneous occurrence of mammary tumours in female rats, (ii) the small number of animals used in the various test groups, (iii) the lack of reference controls or data on natural variations of test parameters, (iv) the lack of data on the agronomic, compositional and phenotypic characteristics of the test and control materials, (v) the use of unconventional statistical methods and (vi) the lack of power analysis prior to the start of the study. The deficiencies noted in this study render any claims of the authors regarding long-term adverse effects of GM maize NK603 highly disputable and scientifically unfounded.

The data presented by Séralini et al. (2012) are all the more surprising because maize NK603, submitted for EU market authorization, has previously been assessed by the EFSA GMO Panel in 2003 and 2007 (EFSA GMO Opinions, www.efsa.europa.eu/en/panels/gmo.htm). These assessments indicated that maize NK603 was (with the exception of the presence of the newly expressed proteins CP4 EPSPS and CP4 EPSPS L214P) compositionally and agronomically equivalent both to an appropriate non-GM maize variety (with a comparable genetic background) and to other conventional maize varieties. Furthermore, a 90-day study in rats fed with either maize NK603 (as 11% or 33% of the diet) or non-GM maize grain with a comparable genetic background indicated no consistent differences in clinical, biochemical and histological parameters, except for slightly elevated levels of average corpuscular volume and haemoglobin in female rats at the high dose, which were not considered as biologically relevant.

Is there a need for additional data requirements for the risk assessment of foods/feed derived from GM plants?

An important issue in the safety/nutritional evaluation of GM foods/feed is the assessment of unintended effects caused by the genetic modification, which may lead to compositional alterations with potential toxicological effects on humans/animals. It is generally accepted that perturbations in the plant’s physiology and biochemistry will likely be detected by phenotypic, agronomic or targeted compositional analyses. The potential for unintended effects is not confined to GM crops but will apply to all breeding technologies including conventional crossing. Examples are well documented (Cellini et al., 2004). So called ‘traditional’ or ‘conventional’ breeding will produce intended or unintended mutations, deletions, insertions and rearrangements in the genome, but the history of safe use of products developed with these approaches tells us that few toxicologically hazards (e.g. enhancement of levels of natural toxins) have arisen.

To detect unexpected (unintended) changes in crop composition, targeted chemical analyses of the GM plant and its derived food/feed are performed and compared with that of the appropriate non-GM controls. A broad array of macro- and micronutrients, antinutrients and natural toxins are included in the analyses and the OECD has designed crop-specific compounds to be monitored (OECD Consensus Documents, http://www.oecd.org/ehs). The extensive array of compounds analysed, which represent many relevant metabolic pathways, enables the risk assessor to identify with confidence the possible occurrence of unintended effects. However, it is clear that complete assurance cannot be provided due, for example, to bias in the selection of compounds, to differences in the efficiency of extraction methods and availability and sensitivity of detection methods and to the lack of resolution power for some of the compounds assayed (for example the quantification of total proteins rather than proteome analysis).

Over the years, data generated on comparative compositional analyses of GM plants and derived food/feed and submitted in the framework of the EU regulation on GMOs have not indicated that the genetic modification of plants results in significant unintended alterations that would impact on safety (EFSA GMO Opinions, www.efsa.europa.eu/en/panels/gmo.htm). Similarly, a recent review of data on compositional analyses of GM plants compared to traditionally bred plants, which includes cases evaluated by the US FDA and Japanese authorities, indicates compositional equivalence (Herman and Price, 2013). Moreover, variations in composition resulting from traditionally bred crops under different environmental conditions were much larger than alterations resulting from transgenesis. The authors raise the question as to whether the compositional equivalence studies
required for GM crops are still justified on the basis of scientific uncertainty.

A broader scale and less-biased analysis of the composition of GM plants compared with non-GM plants is nowadays possible without specific selection of compounds. Indeed, ‘omics’ technologies in general provide wide coverage of genes (transcriptomics), proteins (proteomics) and metabolites (metabolomics) and a growing number of examples indicate that such profiling approaches are useful in identifying sources and extents of variation in the composition of crops, including unintended effects of breeding techniques. It has been well demonstrated that such variation is caused, for example, by genetic background, breeding technique used, growing environment (site, season), genotype–environment interactions and crop cultural practices (Barros et al., 2010; Davies et al., 2010; Kok et al., 2008; Ricroch, 2013). A number of studies indicate that the genetic modification induces minor changes in plant gene expression and composition, whilst environmental factors have a much greater impact. The value of these technologies during product development is obvious. Similarly, these technologies will likely contribute to the identification of unintended effects, but their usefulness in routine risk assessment remains to be established.

As indicated earlier, EFSA recommends 90-day feeding trials with whole food/feed derived from GM plants only under certain conditions, whilst the new implementing regulation of the EU demands that these experiments are routinely carried out for all GM applications related to single transformation events and, where appropriate, for GM plants containing stacked transformation events. As an apparent justification for the obligatory requirement, it is stated that ‘It has, however, not been proved possible to define with the necessary precision the level of uncertainties which would require the submission of 90-day feeding studies’. This argument is curious, because EFSA has indicated that on a case-by-case basis, the performance of 90-day studies may be considered when uncertainties identified in the course of the risk assessment justify the need.

The new EU requirement for routine testing of food/feed derived from GM plants in 90-day studies is not based on sound science but rather satisfies political agendas of some regulatory agencies which are of the view that issues, other than scientific ones, need to be taken into account (e.g. socio-economic). However, it is remarkable that such pseudo-scientific arguments are introduced into the scientific risk assessment process in the EU. It discredits both the sound scientific risk assessment approaches developed for GM foods and the risk assessors involved. Moreover, it is disproportionate as it fails to take into account, on a case-by-case basis, the source and nature of the vast majority of plant substances/toxicants that are potentially consumed on a daily basis. Of particular concern is that routine animal testing will lead to an unnecessary waste of animals, which is contradictory to EU Commission policies to stimulate the RRR principles, that is, replacing of animal tests by alternative methods, refine methods to reduce pain and distress of animals and to reduce the number of animals wherever possible (European Directive 2010). Furthermore, it creates false expectations for the consumer regarding a possible reduction in uncertainties associated with the risk assessment of GM foods and will isolate the EU in the international risk assessment arena. This may have further negative consequences for global trade.

In conclusion, current approaches for the safety and nutritional assessment of foods/feed derived from GM plants as developed by national and international bodies like OECD, FAO/WHO, and EFSA, have proven to be proportionate and fit for purpose. They allow flexibility and facilitate the inclusion of the latest scientific insights and guarantee a high level of safety of these products once a positive evaluation has been completed. Thus, there is no need for additional requirements, that is, routine (sub)-chronic testing of foods/feed derived from GM plants.
References


EFSA. (2011b) EFSA Scientific Committee Opinion Guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA J. 9, 2438–2459.

EFSA (2012) Final review of the Séralini et al. (2012a) publication on a 2-year rodent feeding study with glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology. EFSA J. 10, 2986–2996.


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