90-day rodent feeding studies on whole GM food/feed

Is the mandatory EU requirement for 90-day rodent feeding studies on whole GM food/feed fit for purpose and consistent with animal welfare ethics?

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Genetically modified (GM) plants and derived food and feed products are subject to risk assessment and regulatory approval before these can be marketed in the European Union (EU). This pre-market risk assessment follows international standards to evaluate whether characteristics of the GM plant associated with the genetic modification are capable of causing adverse effects on human and animal health and the environment (hereafter referred to as hazards) [1]. Such hazards can arise directly from the novel trait intentionally introduced in the GM plant, or from unintended changes (Sidebar A). As unintended changes are, by their nature, generally unexpected, it is difficult to foresee them based on a specific risk hypothesis and test for them directly [2]. The identification of unintended changes in GM plants is therefore based on the molecular, agronomic/phenotypic and compositional characterisation of the GM plant (Sidebar B) [1,2]. Any unintended changes identified are then assessed further for their potential to cause harm to human and animal health or the environment.

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Since 8 December 2013, the Implementing Regulation (EU) No. 503/2013 provides detailed instructions for the risk assessment of GM food/feed in accordance with Regulation (EC) No. 1829/2003. As part of the toxicological assessment of GM plants, the Implementing Regulation now requires a mandatory 90-day feeding study in rodents on whole GM food/feed in order “to identify potential adverse effects on the whole GM food/feed, or address remaining uncertainties.” The mandatory nature of this requirement introduced a significant shift in the approach to 90-day rodent feeding studies by necessitating such a study even when no specific risk hypothesis can be formulated, that is without any evidence on changes or hazards identified in prior studies [3]. The Implementing Regulation was endorsed by a qualified majority of EU Member States after a lengthy discussion between Member States and the European Commission, and aims to incorporate the existing guidelines of the European Food Safety Authority (EFSA) into a legal text [1].

Yet, the mandatory requirement for a 90-day rodent feeding study is not unanimously accepted on scientific grounds by various stakeholders. There is still a considerable debate with widely diverging positions among citizens, academia, risk assessment bodies, industry and non-governmental organisations [4,5]. Some stakeholders argue that feeding studies on whole GM food/feed are not sensitive enough to detect adverse effects without a risk hypothesis that would allow tailoring the study design to the

suspected safety concern. Moreover, as those studies are unlikely to yield meaningful results relevant to safety, their mandatory conduct creates a dilemma for animal welfare.

“Given the inherent limitations of untargeted 90-day rodent feeding studies [...], their mandatory conduct is inconsistent with the EU policy goal of improving animal welfare in scientific research and regulatory testing”

Others argue that unintended changes that might cause adverse effects are unlikely for GM plants that enter the regulatory review process, because such changes would have been discarded by plant breeders during development. They further assert that no credible reports of unanticipated adverse effects on human and animal health in GM plants have yet been published. Consequently, safety assessment should focus on known hazards associated with the novel traits, such as the expression of insecticidal proteins to control pests or compositional, metabolic or physiological changes in the GM plant, in order to evaluate plausible risks instead of unknown ones (Sidebar C). Other stakeholders, however, claim that molecular, agronomic/phenotypic and compositional data are not sufficient to detect unintended changes with sufficient confidence to forego animal feeding studies.

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Sidebar A: Definition of unintended effects

The following definitions of unintended effects are used internationally:

- Canadian Food Inspection Agency (CFIA): In addition to intended changes caused by the insertion/mutation of defined DNA sequences during preparation of a GM plant, additional unintended traits may be acquired or existing traits could be lost or modified. These unintended effects may be deleterious, beneficial or neutral with respect to health of plant and safety to livestock and humans.
- Codex Alimentarius: In achieving the objective of conferring a specific target trait (intended effect) to a plant by the insertion of defined DNA sequences, additional traits could, in some cases, be acquired or existing traits could be lost or modified (unintended effects). The potential occurrence of unintended effects is not restricted to the use of *in vitro* nucleic acid techniques. Rather, it is an inherent and general phenomenon that can also occur in conventional breeding. Unintended effects due to genetic modification may be subdivided into two groups: those that are “predictable” and those that are “unexpected”. Many unintended effects are largely predictable based on the knowledge of the inserted trait and its metabolic connections or of the site of insertion.
- European Food Safety Authority (EFSA): Unintended effects of the genetic modification are considered to be biologically relevant differences between the GM plant and the appropriately selected comparator(s), which go beyond the primary intended effect(s) of the genetic modification.
- Food Standards Australia New Zealand (FSANZ): In addition to the intended effect (e.g. a new trait, such as insect protection), there may also be other effects associated with the genetic modification that were unintended (e.g. compositional changes to the food) and which may impact on the health and safety of the population. Therefore, it is important that both the intended and any unintended effects are evaluated. Unintended effects are not restricted to the use of gene technology, but can also occur with the use of conventional techniques of genetic modification such as traditional breeding.

Before the Implementing Regulation went into effect, EFSA considered the necessity to perform 90-day rodent feeding studies on a case-by-case basis, based on the relevant changes or hazards identified in the preceding molecular, agronomic/phenotypic and compositional characterisation of the GM plant [1,6]. The mandatory requirement for 90-day rodent feeding studies raises several challenges in regard to the objectives and performance of such studies. The first is that the study has to fulfil two separate objectives: to support the identification of potential adverse effects in whole GM food/feed in the absence of any relevant changes or hazards identified in the preceding analyses (representing an *untargeted* exploratory study), or to address relevant changes or specific hazards identified in previous analyses of the GM plant (representing a *targeted*, hypothesis-driven study) [3,7].

The protocol for 90-day rodent feeding studies on whole GM food/feed derives from the OECD (Organisation for Economic Co-ordination and Development) Technical Guideline for the “Repeated Dose 90-day Oral Toxicity Study in Rodents, 408” for testing chemicals and has been adapted to the complex matrix of whole food/feed. Despite these adaptations, the use of whole food/feed as test material introduces limitations. The highest achievable dose is limited by the

satiety of the animals and the need to provide nutritionally balanced diets. Consequently, achievable dose levels may not be sufficient to identify hazards and might be too low to simulate realistic exposure for humans and animals [7]. Substances with a low toxic potential that are present in small amounts in food/feed would be below the no-observed-effect level at normal intake, and therefore unlikely to cause any observable effects in untargeted 90-day rodent feeding studies [6]. Another limitation is that, in the absence of a clearly articulated risk hypothesis, it is not possible to define an effect size of toxicological relevance *a priori* and thus an appropriate sample size to ensure sufficient statistical power [7]. EU risk assessors are therefore faced with the challenge of how an untargeted exploratory study should be interpreted within the frame of the toxicological risk assessment of GM plants [3].

In the light of the mandatory requirement for 90-day rodent feeding studies, EFSA provided more detailed instructions on how to apply the existing EFSA guidelines [1,6,8] to support the design and interpretation of targeted 90-day rodent feeding studies on whole GM food/feed [7]. It also considers an alternative scenario, in which untargeted 90-day rodent feeding studies are conducted in the absence of identified hazards [7].

The second challenge is the legal and public pressure to limit the use of animals in scientific research and regulatory testing. Directive 2010/63/EU makes it a legal requirement to replace, reduce and refine (3Rs) the use of animals in research. The 3R approach advocates to use less animals in experiments, plan and refine their use in such a way that pain and distress are minimised, and replace higher animals with alternative methodologies and lower organisms where feasible. Given the inherent limitations of untargeted 90-day rodent feeding studies on whole GM food/feed, their mandatory conduct is inconsistent with the EU policy goal of improving animal welfare in scientific research and regulatory testing [4,5].

The third challenge is that the mandatory requirement for 90-day rodent feeding studies is not consistent with the international consensus among risk assessment bodies. In most jurisdictions, molecular, agronomic/phenotypic and compositional data are regarded as sufficient to test risk hypotheses of no difference between a GM plant and its comparator. The new mandatory EU requirement therefore potentially hampers the global harmonisation of data requirements.

E FSA has made several efforts to design a robust approach for the risk assessment of unintended changes in GM plants at EU level [1]. EFSA gave special attention to: the selection of appropriate comparators for the agronomic/phenotypic and compositional characterisation of GM plants, the use of study-specific reference varieties to facilitate a reliable estimation of natural variability in agronomic/phenotypic and compositional characteristics, and the design of agronomic/phenotypic and compositional field trials and the statistical analysis of the data gathered.

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Beyond those recommendations, there are several potential avenues that could be taken to further improve the assessment of

Sidebar B: Identification of unintended changes

Risk assessment typically follows a comparative approach based on the data derived from the molecular, agronomic/phenotypic and compositional characterisation of GM plants to identify potential hazards. Molecular analysis assesses whether the inserted DNA disrupts endogenous plant genes and whether the GM plant produces proteins other than the intended ones. Agronomic/phenotypic and compositional data determine whether the GM plant has different agronomic/phenotypic characteristics and composition—such as important nutrients, anti-nutrients, toxins and allergens—compared with a conventional counterpart (comparator) grown under similar conditions, and whether the observed changes fall within the natural range known for the plant. The choice of which agronomic/phenotypic characteristics and components to examine is, in part, determined by their ability to cause harm, but is generally broader to allow for the identification of unintended changes in the GM plant. If such unintended changes are identified, they are assessed further for their potential to cause harm to human and animal health. Risk associated with the identified hazards is then characterised by testing hypotheses about the likelihood and severity of adverse effects.

Sidebar C: Technology- versus product-based GMO legislation

The evidence and efforts needed to inform the assessment of potential adverse effects on human and animal health caused by unintended changes in GM plants and derived food/feed products remain a contentious issue of debate in some jurisdictions. A recurring comment is that unintended changes in plants are not unique to genetic modification and that their assessment should be considered within the context of those caused by other plant breeding methodologies, such as mutagenesis. Some stakeholders have therefore questioned why more stringent requirements apply to genetic engineering, given that other plant breeding techniques might result in similar or even larger genomic alterations. Instead, they argue that product-based legislation would enable a more consistent and proportionate approach to risk assessment of plants with similar novel traits, irrespective of the technologies used.

unintended changes in GM plants. The first approach would be fine-tuning the choice of plant components that are examined during the compositional characterisation. Currently, the selection of components follows the detailed lists of plant-specific components outlined in the various consensus documents of the OECD. These components should represent the plant's metabolism and function, and maximise the probability of detecting any unintended changes. However, additional international efforts could prioritise components based on their ability to predict harm and capture relevant changes [9]. The experience gained during the past 20 years could help to revise the compositional parameters for each plant species, taking into account their relevance, variability and nature.

A second avenue would rely on advances in genomics and bioinformatics to develop alternative testing approaches to support the hazard identification of food/feed safety assessment of GM plants. High-throughput, next-generation sequencing offers unprecedented possibilities for detailed comparisons of genomes [10], along with parallel advances in high-throughput analysis of gene expression (transcriptomics), protein expression (proteomics) and metabolite

composition (metabolomics). These methods have great potential to increase our knowledge of plant physiology and metabolic networks, and may improve targeted analyses [9]. Such profiling techniques could be an alternative tool to identify unintended changes in the composition of test materials, when for instance specific metabolic pathways have been intentionally modified [5]. This information may contribute to a better understanding of unintended changes in plants caused by genetic engineering, and usher in a more targeted, hypothesis-driven approach to search for relevant unintended changes.

In conclusion, the mandatory EU requirement for 90-day rodent feeding studies to assess unintended changes in GM plants relevant to human and animal safety is not unanimously accepted on scientific grounds. Several stakeholders argue that: untargeted feeding studies are too insensitive and unlikely to yield meaningful results relevant to safety; not in compliance with the EU policy goal to replace, reduce and refine animal testing; and potentially hampering global harmonisation of risk assessment requirements. EFSA guidelines recommend a case-specific and hypothesis-driven approach

for 90-day rodent feeding studies. The necessity to perform such studies should be triggered by preceding molecular, agronomic/phenotypic and compositional characterisation of GM plants. The risk hypotheses formulated will then tailor the study design to ensure that animal feeding studies, including those on whole GM food/feed, are conducted when needed, and that animals are not sacrificed needlessly.

The EU FP7-funded research project “GMO Risk Assessment and Communication of Evidence (GRACE)” recently published preliminary results of 90-day rodent feeding studies and a 1-year rodent feeding study (<http://www.grace-fp7.eu/>), and concluded that rats are not adversely affected when fed GM insect-resistant maize MON810 at a level of up to 33% in the diet. Based on the evidence, the GRACE consortium concluded that their findings support the scientific reasoning that 90-day rodent feeding studies on whole GM food/feed are only meaningful if a clear risk hypothesis can be formulated based on a specific hazard identified in the preceding molecular, agronomic/phenotypic and compositional characterisation [5].

Article 12 of the Implementing Regulation includes a review clause: “the Commission shall review the requirement to perform 90-day feeding studies in rodents with whole genetically modified food/feed [...] on the basis of new scientific information.” Furthermore, the Implementing Regulation states that “the Commission shall in particular monitor the outcome of the research project called GRACE under the 2012 work programme of the seventh Framework Programme for Research (FP7).” The scientific value and limitations of animal feeding studies ranging from 90 days to 2 years with whole GM food/feed (maize MON810 and herbicide-tolerant NK603, treated and untreated with the intended herbicidal active substance glyphosate), are currently being further investigated (<http://www.g-twyst.eu/>; <http://www.rechercheriskogm.fr/en/article/gmo90-research-project-progress>). Data from the G-TwYST and GMO90+ research projects will become available during 2017 and 2018, respectively, and can inform discussions on how to improve safety testing of GM plants while reducing the need for animal testing.

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Conflict of interest

The authors declare that they have no conflict of interest.

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