RAGES

RISK ASSESSMENT OF GENETICALLY ENGINEERED ORGANISMS IN THE EU AND SWITZERLAND

Summary report of the results from the RAGES project

2016-2019

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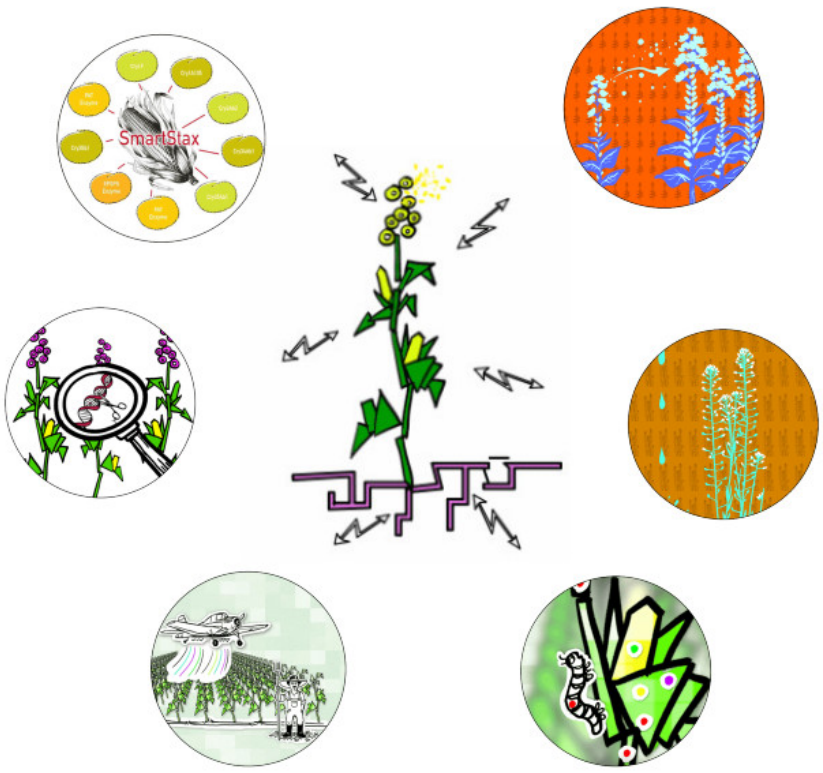

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Summary

The RAGES project (Risk Assessment of Genetically Engineered Organisms in the EU and Switzerland) evaluated the risk assessments of genetically engineered (GE) food plants (also known as genetically modified organisms, GMOs) currently performed by the European Food Safety Authority (EFSA). RAGES focused on the risks of transgenic plants intended for food production, and also takes into account some new methods of genetic engineering (genome editing). Partners in the project are the European Network of Scientists for Social and Environmental Responsibility (ENSSER), its Swiss branch CSS (Critical Scientists Switzerland), GeneWatch UK and Testbiotech.

The RAGES project findings show that EFSA, the EU Commission and, consequently, also the Swiss regulatory authorities, are repeatedly failing to achieve what is required by legislation. While official rhetoric proclaims that the system fully protects public and environmental health from the risks arising from the cultivation and/or consumption of foods and feeds derived from GE plants, in reality current orthodox risk assessment policies and practices are compromising the protection of public and environmental health. Instead of prioritising the protection of human, animal and environmental health, the EU gives priority to the interests in developing and marketing of GE organisms.

An implicit question which underlies all scientific risk assessment, whatever its object, is about how the crucial questions have been initially defined, or framed. This choice is not only a purely scientific question, but also a policy one – and as a responsibility for the risk manager, it arises prior to scientific analysis (see Codex Alimentarius Commission, 2004; Millstone, 2007; Millstone et al 2008). For example, if the risk question is framed narrowly, this may allow greater precision in answering the questions, but at the expense of failing to recognize and attend to potentially serious harm and adverse effects. Our findings show that despite years of constructive criticism to this effect, both EFSA (as risk assessor), and the European Commission (as risk manager) have chosen to overlook the consequences of a too narrow framing in risk assessment policies (RAPs) for the implementation of the Precautionary Principle.

The GE organisms RAPs that would dovetail most readily with the legislative mandate within which EFSA and the Swiss authorities are supposed to operate, would be ones that no longer tolerated the unduly narrow framings that have been adopted and defended by EFSA's GMO Panel[1] in particular. For example, one such framing involves pretending that the total effect of any novel GE plant, or foodstuff derived from it, is fully predictable from a consideration only of the sum of the possible effects of each of its individual compounds, as if they could only ever act independently of all others. Currently, from this implicit reductionist framing, small amounts of what are inadequate and fragmentary data are being combined with large amounts of wishful thinking to produce reassuring scientific narratives of 'no harm', while the limitations of knowledge are mostly neglected along with their uncertainties.

These problematic aspects of the EFSA GMO panel's implicit and unaccountable risk assessment policies, which the RAGES project has revealed, indicate that those risk assessments are far more likely to under-estimate the range and severity of possible adverse effects rather than to over-estimate them. In addition to failing fully to comply with existing EU law, this practice is also incompatible with the European Union's policy commitment to the Precautionary Principle. Multiple and systematic failures (by default) to adopt a precautionary approach are evident in every one of the RAGES sub-sectoral reports. EFSA's practices have therefore been contrary to the

1 Name of EFSA's expert panel that assesses the risks of GE organisms

provisions of the legislation that stipulate: "…a scientific evaluation of the highest possible standard… of any risks which they present for human and animal health and… for the environment."[2]

Scientific risk assessments may be precautionary if they are comprehensive, or they will be permissive if they are incomplete. The European Commission's risk managers need to ensure that the EU's precautionary policy commitments, as well as the legislative requirements for including long-term, cumulative and indirect risks, are properly implemented in EFSA's risk assessments, as well as when Commission policy officials decide how putative risks are to be managed. The same is true for Swiss authorities and for Swiss government decision-making.

The outcome of RAGES

- makes explicit many flaws and gaps in current risk assessments,
- specifies particular needs for further research and
- provides recommendations for enhancing current standards and giving more weight to the Precautionary Principle.

Many of the changes that need to be made will have to be implemented by and within EFSA or the counterpart Swiss competent authorities, but the findings of the RAGES project also have important implications for the European Commission, and the Swiss government, which act as the responsible risk managers and policy decision-makers. For example, specific provisions in EU legislation stipulate some particular testing and data requirements for applicants for authorisation to market GM food or feed.[3] While those requirements may need to be changed, EFSA (and similarly the Swiss risk assessors) are not empowered to enact such changes, since they are supposed not to be policy authorities.

The recommendations are intended to enhance the scientific and democratic legitimacy of policy procedures and outcomes, and to ensure that the explicit objectives of European legislation are achieved.

2 See REGULATION (EC) No 1829/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2003 on genetically modified food and feed, Official Journal of the European Union L 268/1-23, October 18, 2003, Recital 9, https://eur-lex.europa.eu/legal-content/DE/TXT/PDF/?uri=CELEX:32003R1829&from=en

3 COMMISSION IMPLEMENTING REGULATION (EU) No 503/2013, https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32013R0503

1. Introduction

The RAGES project (Risk Assessment of Genetically Engineered Organisms in the EU and Switzerland) has critically evaluated risk assessments of genetically engineered (GE) food plants as performed by European Food Safety Authority (EFSA) and its Swiss counterpart. RAGES focused on the risks of transgenic plants intended for food production, and has also taken into account some new methods of genetic engineering (genome 'editing').

Partners within the project are the European Network of Scientists for Social and Environmental Responsibility (ENSSER), its Swiss branch CSS (Critical Scientists Switzerland), GeneWatch UK and Testbiotech. The project was funded by the Mercator Foundation Switzerland.
The project is completely independent of the interests of the biotechnology industry. It aims to improve overall food and environmental safety and enhance scientific understandings of risks and potential hazards caused by the introduction of GE plants into ecosystems; in addition, it provides a more precise understanding of how EFSA scientific risk assessments have been framed and conducted.

In this report, the precise terminology was also part of the discussion: instead of the more common EU language using the term 'GMO' (genetically modified organism), we mostly preferred the term 'genetically engineered (GE) organism'. This is because the term 'GMOs' also includes categories of organisms such as plants derived from so-called 'random mutagenesis', which are not subjected to the EU approval process and therefore no mandatory risk assessments of those categories of organism is required. By referring to GE organisms, it is clear, that the organisms we are concerned with, are subjected to the approval process under EU Directive 2001/18/EC, including the new methods of genetic engineering such as so called genome 'editing'. While in some of our technical reports the term GMO might still be used, especially when citing other authors and documents, it is meant to be understood as 'GE' as defined above.

Within the EU and Switzerland, GE organisms are matters of societal controversy, especially if released into the environment or used for food production. In this context, the identification and determination of risks, potential hazards and the likelihood for adverse effects are of utmost importance. But currently, official discussions about risks are largely dominated by the perspective of the agbiotech-industry. Those companies are funding and controlling most research projects on transgenic plants and are generating the data which inform the approval process. They also exert considerable influence on regulatory authorities and political decision making. At the same time, they try to give the impression that all the risks of GE organisms are strictly controlled, and that the safety of their products has been demonstrated. In consequence, risks are denied and relevant findings are overlooked by the current regulatory system, as for example recently shown by Ferment et al., who showed that a corpus of no fewer than 750 studies, which indicate potential risks from GE organisms, have been disregarded by statutory regulatory bodies (Ferment et al., 2017).

More specifically, in regard to the work of EFSA's GMO Panel, a recent study (Chvátalová, 2019) comes to the conclusion that

> *"Studies are often misquoted, using a wrong citation or representing the results and methods imprecisely. Information from the studies is used selectively, in particular leaving out negative effects and further research requirements. (…) Similarly, EFSA does not communicate any uncertainties, although these are sometimes explicitly stated in the cited literature, and does not provide its own reflection on the relevance of the results under*

natural conditions. Moreover, the cited information is not comprehensive, as evidenced by additional relevant articles found through a literature search but not used in the Opinion."

In this publication, Chvátalová (2019) examines environmental risk assessment of maize MON810 using the example of honeybees and earthworms. Chvátalová (2019), similar to RAGES, which was working on a broader range of examples, comes to the conclusion that

"*the body of referenced evidence is insufficient to draw conclusions on risk*".

Furthermore, crucial

"*conclusions of the GMO Panel are inappropriate and misleading.*"

RAGES experts agree that these problems should no longer be neglected; their continued neglect is scientifically indefensible and politically unsustainable. Against this backdrop, RAGES aims to bring together the perspective of European scientists who focus on and prioritise the goal of protecting health and the environment.

The outcome of RAGES: 1) makes explicit many flaws and gaps in current risk assessments, 2) specifies particular needs for further research and 3) provides recommendations for enhancing current standards and giving more weight to the Precautionary Principle.

The project also involved contributions from experts from regulatory authorities from the EU and Switzerland. Their participation did not aim at achieving overall consensus. But in many cases, the discussion helped to refine our analyses, to supplement further information and to test specific hypotheses during several stages of the project. Therefore, we would like to thank especially the experts from EFSA and from the EU Commission for their participation and their input, without claiming that any findings reported by RAGES represents the point of view of those institutions. We also thank the Mercator Foundation Switzerland for funding this project and being extremely patient concerning the overall duration of the project, which was necessary to take on board the different points of view.

The analyses performed by the RAGES consortium are based on case-studies derived from published opinions of EFSA, peer reviewed publications, and additional scientific data/expertise. Based on this material, RAGES produced six reports on specific topics that were identified as being crucial in this context.

These topics are:

- health risks associated with the consumption of products derived from herbicide tolerant GE plants;
- the assessment of environmental risks associated with the cultivation of insecticidal Bt crops;
- health risks associated with the consumption of products derived from GE plants with altered nutritional composition;
- health risks associated with the consumption of products derived from GE plants with a combination of traits;
- environmental risks from the persistence, self-propagation and uncontrolled spread of GE plants; and
- risk assessment of GE organisms derived from new genetic engineering technologies.

This summary report gives an overview on the findings in the sub-reports as well as on more general considerations and relevant cross cutting issues.

The RAGES reports, as developed from the perspective of the public and environmental health protection goals, provide a unique source of material and reliable information for the public, political decision-making, scientific institutions and experts dealing with the risks of GE plants.

Some of the results will be forwarded for further review in scientific journals. A first peer reviewed publication was accomplished in December 2019 (Miyazaki et al., 2019), another peer reviewed publication on risks of glyphosate resulted from the wider context of the project (Bohn & Millstone, 2019).

2. Overview of the regulatory framework of the current approval process

In the EU, the European Food Safety Authority (EFSA) is responsible for risk assessment of GE organisms. EFSA, in particular its GMO panel, assesses applications for approval of GE organisms for import (for production of food and feed) as well as for domestic cultivation. The most relevant legal frameworks for risk assessment in the EU are EU Directive 2001/18/EC and Regulation (EC) No 1829/2003. In addition, Commission Implementing Regulation (EU) No 503/2013 defines standards for health risk assessment of food and feed products derived from GE plants.

In Switzerland, several authorities are involved in the assessment of applications for the approval of GE organisms but, in most cases, their findings and conclusions do not differ from those of EFSA. While there has been a moratorium on the cultivation of GE plants since 2005, several of the applications for import were approved in Switzerland, based on the same set of data as used by the EU when it decided to approve their importation. Even though there are no officially-recorded imports into Switzerland of food and feed derived from GE plants, researchers found GE oil seed rape growing spontaneously along transport routes (Schoenenberger & D'Andrea, 2012). This shows that Switzerland should continue to follow the debate on import or cultivation of GE plants closely. It is likely that in the future Swiss risk management policies on GE organisms will also be influenced by the standards and practices of the EU.

The legislation under which the EU regulates risk assessment of GE organisms stipulates a high level of protection for health and the environment. Article 4 (and Article 16) of the Regulation (EC) No 1829/2003, requests that, for food and feed products derived from GE organisms, it is "*adequately and sufficiently demonstrated*"

"*must not: have adverse effects on human health, animal health or the environment*".

This includes ensuring that:

> *"…genetically modified food and feed should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken under the responsibility of the European Food Safety Authority (Authority), of any risks which they present for human and animal health and, as the case may be, for the environment."* [4]

4 See REGULATION (EC) No 1829/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2003 on genetically modified food and feed, Official Journal of the European Union L 268/1-23, October 18, 2003, Recital 9 https://eur-lex.europa.eu/legal-content/DE/TXT/PDF/?uri=CELEX:32003R1829&from=en

That regulation also explains that its objective:

> *"…in accordance with the general principles laid down in Regulation (EC) No 178/2002, is to: (a) provide the basis for ensuring a high level of protection of human life and health, animal health and welfare, environment and consumer interests in relation to genetically modified food and feed…"*[5]

Similarly, a high level of environmental risk assessment (e.r.a.) is requested by EU Directive 2001/18/EC[6], which, for example, in section A of Annex II states that:

> *"The objective of an e.r.a. is, on a case by case basis, to identify and evaluate potential adverse effects of the GMO, either direct and indirect, immediate or delayed, on human health and the environment which the deliberate release or the placing on the market of GMOs may have."*

There is ongoing controversy over whether or not the risk assessments as performed by EFSA are adequate to comply with those requirements. For example, within recent years the EU Parliament has adopted around 40 resolutions against further EU approvals. This in itself raises doubts about the scientific standards at EFSA[7]. Some member states' scientific authorities have also objected to EFSA risk assessment Opinions approving GE organisms. Nevertheless, based on the opinions of EFSA, around 80 events[8] of GE plants are currently approved for import into the EU. In addition insecticidal maize MON810 is grown in a small number of EU member states, especially in Spain. The number of GE plants authorised in Switzerland is much lower than in the EU.

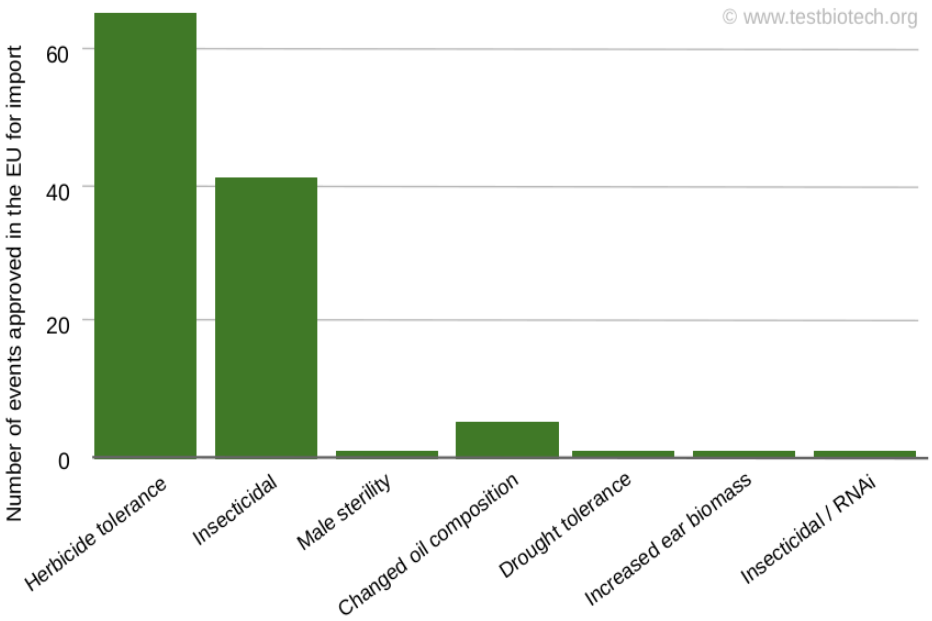

Figure 1: Number of events approved in the EU for import, categorised in traits; the overall number of events authorised up until December 2019 was around 80. (Many events inherit a combination of traits, therefore the number of traits is higher compared to the number of events).

5 Op cit Article 1 p. 268/6

6 DIRECTIVE 2001/18/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC, https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32001L0018

7 www.greens-efa.eu/files/doc/docs/e491b1b487e5c6b48f553e1ef027bccf.pdf

8 An "event" characterized by the gene construct and its place of insertion in the plants genome

3. General overview and cross cutting issues

While assessing the six specific topics, we also identified more general and conceptual problems in current risk assessments as performed by EFSA. Some of these problems are rooted in the reductionistic approach adopted by EFSA. Others are cross-cutting issues, where recent findings in several fields of biology are not yet acknowledged or addressed by EFSA.

3.1 Holistic versus reductionistic approach

Several of the conceptual problems identified in the work of EFSA are consequences of a simplistic, reductionistic and mechanistic scientific model of risk causation and risk assessment, which fails to include the real complexity that is fundamental to all biological systems on many levels, in DNA, cells, organisms, and ecosystems and their interactions.

For example, a common practice in risk assessment of GE organisms is to divide them into parts and individual chemical compounds, and to assess the effects of a few of those parts in isolation from each other, or the environments into which they will be released. A fundamental assumption of this approach is that the functioning of organisms can be fully deduced from the functions of their separate parts, based on a mechanistic concept of chemical compounds and reactions that can be assessed in isolation of each other. This approach tries to reduce complexity to something that can legitimately be subjected to narrowly-designed risk assessments. But the results of this do not reflect the reality they purport to assess; they fail to acknowledge the true levels of uncertainties and gaps in knowledge, thus harmful consequences may be overlooked. This approach is functional in the limited sense that it is designed to appear precise and controlled, and to grant fast track approvals; but it largely ignores the Precautionary Principle, in part because it fails to acknowledge the limits of the available knowledge or key uncertainties.

This current practice of EFSA can be called a “restrictive evidential culture” of risk assessment (Böschen, 2009). That approach can be contrasted with a “holistic evidential culture”, which arguably would be better suited because it would acknowledge various types of uncertainties and ignorance, and would also acknowledge the prior framing choices that 1) define what is deemed to be a relevant risk, 2) define what is deemed to be relevant, necessary and sufficient evidence, and 3) influence the ways in which available data are interpreted. According to Böschen (2009), these two cultures of risk assessment on GMOs can be contrasted as follows (page 513: “Control-oriented epistemic cultures proceed in a restrictive–experimental way and are oriented towards an improvement of (technological) options for action. In contrast, complexity-oriented epistemic cultures structure their knowledge in a holistic–contextual way and enhance options for reflection… these cultures generate knowledge relevant for making decisions, but they do not find a balanced attention in the risk policy of the GMOs…”.

As the RAGES project shows, EFSA and the EU largely follow the approach of the “restrictive evidential culture” which is not adequate in the face of the non-predictable, non-linear nature of life forms and the emergent properties of new combinations and interactions.

3.2 Some differences between genetic engineering and conventional breeding

Risks from GE organisms differ from those associated with organisms derived from natural evolutionary processes, or from processes of conventional breeding. This can be exemplified by taking a closer look at plant breeding: essentially, conventional breeding is always drawn from a

range of genetic and biological diversity found in natural populations, as well as previously bred plant and animal varieties and breeds. In addition, new spontaneous mutations occur and specific triggers can speed up the occurrence of such mutations. In particular with plants, additional ‘tricks’ can be used to increase genetic diversity, e.g. by exposing the seeds to specific chemicals to increase the natural rate of mutation. This process is known as mutation breeding (mutagenesis), which, in a first step, can enhance genetic diversity (Oladosu et al., 2016). Plant cells can also react to non-specific external stress factors. The process of conventional mutagenesis has been used in plant breeding since the mid-twentieth century. It is important to understand that, taken as a whole, the results of mutagenesis are not totally random. They are governed by various biological mechanisms of evolution, inheritance and gene regulation which, for example, ensure that some specific genome locations are more frequently changed than others (Kawall, 2019). The natural mechanisms of inheritance and gene regulation cannot be bypassed with this method. In summary, breeding based on mutagenesis speeds up evolutionary processes that might also occur naturally.

In short, the methods and mechanisms used in what is known as 'conventional' breeding:

- make use of genetic diversity as a starting point;
- are applied to the whole cell or organisms;
- do not insert genetic information using direct technical interventions;
- do not delete genetic information using direct technical interventions

Ultimately, breeding through mutagenesis creates greater genetic diversity, but the desired traits are not brought about by direct technical interventions. It is only through crossing and selection of plants and animals exhibiting desired traits that a new variety can emerge from biodiversity. This process is time-consuming and requires careful choice and repeated testing by breeders. In many cases, unintended effects are eliminated during this long process. Nevertheless, some organisms resulting from conventional breeding might require risk assessment in regard to health and the environment. For example, it is possible to establish herbicide resistant crop plants by means of conventional breeding, which should be investigated in regard to their impact on weedy species and biodiversity (Burgos et al., 2014).

On the other hand, genetic engineering directly intervenes at the level of the genome, i.e. inserting material that was prepared outside of the cells to achieve targeted changes in the genome or epigenome (for further interpretation see the wording of Directive 2001/18/EC, Annex I A)

These techniques and processes:

- are not based on the potential of natural biodiversity and the usage of a large pool of genetic diversity. Rather, the goals of the technical intervention are quite distinct changes in the genome which, in most cases cause specific new gene combinations;
- enable the bypassing of mechanisms of natural heredity and gene regulation;
- enable traits to be established that do not occur naturally, e.g. plants producing insecticidal proteins derived from Bacillus thuringiensis or plants with specific patterns of changes in their genome (as it is often the case with so-called ‘genome editing’ which uses nucleases);
- make it possible to insert additional genes not found in nature: for example, in the case of plants that produce Bt toxins, the DNA sequences are modified in the laboratory giving rise to truncated or chimeric Bt proteins that do not exist in nature (see Hilbeck & Otto, 2015; Latham, 2017).

In summary, experience gained from conventional plant breeding cannot simply be extrapolated to the risk assessment of GE plants. Due to the methods used in genetic engineering, the resulting patterns of genetic change, the resulting gene combinations as well as biological characteristics and

associated risks can be very different compared to those derived from conventional breeding. Thus, according to EU law (Directive 2001/18/EC), all organisms derived from processes of genetic engineering generally require specific, case-by-case risk assessment before they are released into the environment or allowed to be used in food products.

3.3 Some basic challenges in risk assessment of GE organisms

As a starting point for discussion of the risk assessment of GE organisms, it is vital first to consider what is often called the complexity of biology. A short comparison with the risk assessment of chemicals is useful in this context: while chemicals (in many cases) can be considered as clearly defined entities, the characteristics of organisms are largely shaped by their interactions and their mechanisms of self-reproduction, self-organisation and adaptability. Resultant conceptual and practical challenges for the risk assessment of GE organisms can be identified on several levels:

What are the entities and risks that should be assessed?
Life forms can only be fully assessed in combination with their environment: for example, the well established concept of the 'holobiont' (see for example Richardson, 2017 or Sanchez-Canizares, 2017) shows that the biological characteristics of multicellular organisms such as plants, insects or mammals cannot be considered as being completely separated from their associated microbiomes. Organisms and their associated microbiomes interact very closely: it is known that the microbiome can extensively impact the health status of humans, plants and animals (see for example Lynch & Pedersen, 2016). There is evidence that, for example, the maize leaf microbiome composition is not only influenced by environmental factors but to some extent also impacted by the maize plant's genetics (Da Silva et al., 2016; Wallace et al., 2018). Therefore risks can arise, not only from the interactions of GE organisms with their macroscopic, wider environment (such as pollinators and the wood web), but also from interactions with their microscopic, intimate environment (associated microbiomes), and so these interactions should be included in that which needs to be assessed.

How to assess biologically active compounds?
In most cases, physical processes to produce chemical substances can be designed and controlled to produce predictable results. This is not necessarily the case for biological GE organisms: If, for example, new enzymes or insecticidal proteins are produced in GE plants, the mode of action, their efficacy and specificity can be substantially different, when compared with natural variants of their proteins as, for example, originally produced in bacteria (see Hilbeck & Otto, 2015; Latham et al., 2017). Furthermore, due to the insertion or deletion of genes, new open reading frames can occur on the level of the genome that give rise to unanticipated new gene products (such as RNAs), which may be biologically active though unintended and unanticipated processes (see Rang et al., 2004). Therefore, in relation to risk assessments of GE organisms, newly produced gene products should be assessed in the context of the whole organisms and in combination with all other relevant constituents and not in isolation.

How to assess complex cause-effect relationships?
Well defined cause–effect relationships in many cases are not readily identifiable in the case of life forms: as can be shown in GE plants, the interaction of the inserted genes with the genetic background as well as the interactions of the organisms in their environment can play important roles (for references see report in section 4.5). Such multifactorial consequences of genetic engineering are difficult to forecast reliably and so may evade prediction. These interactions can create effects in bidirectional and non-linear ways: it is not only the organisms that impact the environment; it is also that the various environmental conditions, abiotic and biotic stressors, also impact the biological characteristics of organisms. Consequently, risk assessments of GE organisms

should not only assess the impact of the organisms on the full environment, but also vice versa. In addition, possible risks from the resulting combined effects also should be assessed.

How to assess next generation effects?
The characteristics of GE organisms might change from one generation to the next (for references see report in section 4.5). With self-organisation and self-reproduction, and in interaction with changing environmental conditions, next generation effects can occur that may not be predicted on the basis of knowledge about the previous generations. Even if the additionally inserted DNA is transmitted to the next generation in a way that assures genetic stability, this does not mean that the intended function of the gene and the associated phenotype will be transmitted to the offspring as expected. Thus, subsequent generational effects should be considered in all cases where GE organisms could persist and propagate in the environment. This is especially relevant if gene flow occurs from the GE organism into wild populations.

How to take communication and signalling pathways between organisms into account?
Life forms interact and communicate with their environments via multiple bio-chemical pathways. In plants, these pathways for example include exchange of information with other plants, microorganisms and insects (see Schaefer & Ruxton, 2011). Various compounds are involved such as volatile substances, secondary metabolites and biologically active compounds. Environmental risk assessment of GE organisms should include the various ways in which organisms interact and communicate with their environments, taking into account that those environments might not be well characterised in some contexts.

Other specific cross-cutting issues that should be taken into account in risk assessments of GE plants, which either partially or wholly escape current risk assessment performed by EFSA, are summarised in Table 1-4 (see Annex).

4. Specific findings of RAGES

Substantial gaps in EFSA's risk assessments are set out in the detailed RAGES reports. Our findings indicate that EFSA's risk assessments of herbicide tolerant genetically engineered (HT GE) crops provides paradigmatic examples of the shortcomings we have uncovered. While HT GE crops are promoted, advertised and marketed as intimately connected crop-chemical packages, for safety assessment purposes these integrated packages are disintegrated and each of the mutually essential components is separately assessed in a decontextualized fashion which misrepresents reality. In addition, EFSA's overall safety assessment of HT GE plants largely ignores the effects from residues of spraying with the complementary herbicide.

Several findings from the sub-reports all point in a common direction, underlining consistently the reductionistic approach of current risk assessment:

- risk assessments of HT GE crops largely ignore the specific pattern of residues from herbicide sprays and their effects on the overall safety of food and feed;
- risk assessments of HT GE crops do not take into account the application of high dosages and repeated spraying of the complementary herbicides, which is the current practice in commercial cultivation. Therefore, the GE plants tested in field trials do not represent the GE plants as approved for import;
- risk assessment of Bt crops ignores the complexities (and uncertainties) of the modes of action of the toxins or their interactions with co-factors and other stressors;

- risk assessment of Bt crops also largely ignores the fact that the selectivity and efficacy of Bt toxins can be modified by changes in their structure that occur when they are produced in the GE plants;
- if traits are combined in GE crops, such as tolerance to various herbicides and/or the production of several Bt proteins (so-called ‘stacked events’), EFSA fails to require the whole food and/or feed and its mixed toxicity to be tested and assessed;
- if several GE plants are mixed in a diet, the cumulative and combined effects and their mixed toxicity are not investigated;
- metabolic pathways are often multifunctional and complex and can affect a plant’s growth or nutritional composition. However, even if a pathway is directly affected by the genetic intervention, EFSA does not require more detailed assessment of the overall effects;
- only a relatively small fraction of the biologically active compounds that are naturally produced by the plants and their composition are considered in EFSA’s risk assessments;
- large parts of relevant health effects, such as reproductive and immune system effects, as well as the impact onto the gut microbiome, are neglected in EFSA’s current risk assessments;
- even though environmental stressors e.g. ongoing climate change that can influence the expression of the inserted gene constructs, such processes and their impacts are not systematically assessed by EFSA;
- only a small selection of relevant geo-climatic conditions and regions representing the countries of cultivation are taken into account in the field trials required by EFSA;
- EFSA’s assessments of impacts on ecosystems and food webs suffers from major gaps in the selection of relevant organisms and also from neglect of relevant pathways of exposure;
- if GE plants can persist, propagate and spread through uncontrolled gene flow, data on the next generation effects are not required and so are not assessed by EFSA.

Some expert contributors to RAGES compared these findings with some of the most recent opinions from EFSA, as published on stacked maize (EFSA, 2019a) and soybean (EFSA, 2019b). In these cases, EFSA applied EU Implementing Regulation (EU) No 503/2013 by applying a slightly different protocol. However, compared to our findings as listed, no significant differences or improvements can be observed. For example, mixed toxicity of whole plant food and feed was still not tested, the reactions of the GE plants to environmental stressors were not investigated systematically, the expression data of the newly introduced proteins were not reliable, the application of the complementary herbicide was not in line with the much more intense spraying used under practical conditions (Testbiotech, 2019a and 2019b).

Several of these findings are also relevant for risk assessment of organisms derived from new methods of genetic engineering (genome ‘editing’) using tools such as CRISPR/Cas. In the near future the commercial sponsors of such organisms can be expected to apply for consent to market their products. Assessing the risks of such organisms will be challenging and complex, even if no additional genes are inserted. Since the nucleases involved are intended to interfere with all copies of targeted gene families, or with different genes by multiplexing (i.e. changing several genes in one event), the pattern of genetic changes (both intended and unintended) as well as resulting gene combinations, biological characteristics and risks can be significantly different from those obtained using conventional breedingIn many cases, the metabolic pathways of the organisms resulting from genome ‘editing’ are likely to be affected to a greater extent compared to those plants that have so far been subjected to risk assessments. In addition, new, serious and uncontrollable risks may be created by the introduction of gene drives by using tools like CRISPR/Cas, meant to genetically engineer natural populations.

In the following sections (4.1 to 4.6), we give an overview on the six sub-reports that are the main outcomes of the RAGES project:

4.1 Assessment of health risks associated with the consumption of products derived from herbicide tolerant GE plants

Herbicide tolerant genetically engineered (HT GE) plants have been engineered to tolerate herbicides and are never grown in commercial agriculture without being sprayed with the relevant herbicide. As the claimed benefits arise from the application of the herbicide rather than from the GE crop plants in isolation, so the risks and safety issues should be considered in combination. The global use of glyphosate has increased dramatically with HT GE plants as a main driver. This is in itself an important environmental problem. Moreover, the increased use of glyphosate represents a selective pressure that accelerates the evolution of glyphosate resistant weeds. As a response, farmers in the USA, Argentina and Brazil have increased, over the last 20 years, their spraying of HT GE soy. The result is that farmers now spray at rates more than twice as high as those originally recommended. Similarly, the number of glyphosate applications has increased from one or two, to four applications per year, which implies more spraying late in the growing season. In addition, the GE plants are made resistant to other complementary herbicides and must be expected to entail mixed residues (cocktails) of toxic chemicals in the food chain.

This promotes higher residues from glyphosate and/or other complementary herbicides in HT GE soybeans, which dominate the global export market, including to Europe, for use in food and feed products. This raises questions about health effects for livestock and consumers.

The basis for risk related research on, and risk assessment of, HT GE plants is plant samples produced in field trials. A key problem is that these plants are sprayed with much lower, i.e. not representative, doses of the complementary herbicides compared to doses that farmers use in commercial production. Using those plant samples to inform risk assessments can therefore lead to wrong conclusions and underestimate the actual risks: firstly the load of residues is much higher in commercially produced plants, and secondly the plants' composition may be altered by a more intense spraying regime. Thirdly, combinatorial effects can arise from interactions between plant constituents and herbicide residues. Such changes may potentially cause health effects such as toxicological, hormonal or immunological reactions at the stage of consumption.

The risks to ecosystems as well as human and animal health arising from glyphosate tolerant GE plants, are in the process of being replicated and exceeded with new 'stacked' HT GE plants that are tolerant to multiple herbicides such as glufosinate ammonium, 2,4-D, dicamba and isoxaflutole, in addition to glyphosate. These herbicides will be sprayed together and result in new and untested 'cocktail' mixes and co-exposure, both in the environment as well as in food and feed. The toxicity of mixes, interactions and combinatorial effects of these substances are difficult to study and remain substantially unknown. Hence, these new HT GE plants cannot be considered safe.

We illustrate with two current case study examples (triple resistant HT GE soy plants intended for import to the European market) how the European risk assessment system, as implemented by EFSA, fails to perform relevant risk assessment of HT GE plants.

We argue that the underlying causes of these flaws in the risk assessments come from a lack of independent research, a lack of relevant data and the separation of the HT GE plant and its co-technology (complementary) herbicide in risk assessment, i.e. the division of the assessment of the plant, performed by the GMO-Panel and assessment of the pesticide, performed by the PPR-Panel.

To demonstrate safety of HT GE plants, the two areas of risk assessment need to be combined to assess the overall risks of the consumption of food and feed products derived from the HT GE plants. This need is reflected in COMMISSION IMPLEMENTING REGULATION (EU) No 503/2013, which states that the field trials with HT GE plants should compare and test plant products with and without the complementary herbicide being applied. Furthermore, potential synergistic or antagonistic effects resulting from the combination of the transformation events should be included, and the data provided for risk assessment should allow scientists to conclude whether the expected agricultural practices could influence the endpoints that were studied. However, in EFSA's current practice of applying Regulation (EU) No 503/2013, there are major gaps in the risk assessments of HT GE plants, with problems similar to those as mentioned above (see for example, EFSA, 2019b; Testbiotech, 2019b).

In conclusion, the current practice of risk assessment for HT GE plants in Europe fails to assess identified and real risks. Thus, the approval process for HT GE plants is, in its current form, both inadequate and misleading. It is a further step in the wrong direction that new stacked HT GE plants are likely to introduce untested herbicide-cocktails into European food chains. With this background, no further HT GE plants should be approved for import based on the current practice. Moreover, events, which are already allowed for import, need to be re-assessed.

4.2 Assessment of environmental risks associated with the cultivation of insecticidal Bt plants

A risk conclusion is typically based on combining information and data obtained from studying the various routes of exposure to a particular stressor and the potential adverse effects those stressors may cause. The stressors in our case are GE plants expressing toxins from the bacteria *Bacillus thuringiensis* (Bt). Exposure is evaluated in terms of quantity and quality of the stressor (concentration of Bt toxins) and duration/type of exposure to this stressor. However, the framing (problem formulation) and implementation of the risk assessment, including the assumptions made, the selection and limitations of the research results available, and the interpretation of these data, are fundamental in determining what risks can be identified and what risks will be neglected right from the start.

We unravel and illustrate how EFSA's narrow interpretation and implementation of the EU regulations for risk assessment fails at its core. Instead of assessing the real, living GE plant within its complex network of ecological interactions in the real world, EFSA limits the focus primarily to the 'added chemical substances', i.e. Bt toxins, and arrives at its conclusions regarding risks based mostly on data produced with Bt toxins isolated from an artificial bacterial surrogate system, rather than the GE Bt plant (except for the occasional test with pollen). Thus, effectively, EFSA is assessing the GE Bt organism, here a plant, as an isolated, single chemical.

The framing (serving as justification) of this narrow interpretation is based on the conceptual model called 'substantial equivalence' (renamed as 'comparative (safety) assessment') which means that a GE plant is treated as nothing more than the original unmodified parent plant with the chemical, here the Bt toxin, added like a spray-on pesticide. The second assumption is that Bt toxins have a single target specific mode-of-action, at least for non-target organisms.

Both assumptions are based either on no science or on outdated science, which results in EFSA's sweeping/generalised safety claims that lack robust scientific evidential support, or are even undermined by scientific evidence to the contrary. Nevertheless, both assumptions form the pillars

for EFSA's assessments of GE Bt crop plants, which treat them like pesticid chemicals and apply standard first-tier protocols developed by the OECD for regulatory safety assessments of synthetic chemical pesticides.

In contrast to EFSA's narrow 'no risk' conclusions, we show that there is a multitude of pathways exposing many organisms, the vast majority of them being non-target organisms, to both the GE plants and their Bt toxins. These pathways extend from terrestrial to aquatic systems along a diversity of exposure routes, including the recent discovery of intergenerational exposure from parent non-target organisms to their offspring. A myriad of non-target organisms are exposed to, i.e. ingest, Bt toxins, either from live or dead Bt plant material or as free Bt toxins leached from live and dead Bt plants, in significant quantities. Furthermore, these Bt toxins are persistently present above- and below ground, throughout the growing season and beyond, including in aquatic ecosystems such as headwater streams running through the agricultural landscapes where Bt crops are grown. Hence, Bt toxins are highly ubiquitous in large amounts in those agroecosystems where Bt crops are grown, i.e. on more than a 100 million hectares. From there, these Bt toxins spread to aquatic systems via water transport processes within the soil or from water runoff from fields under Bt crop production.

We further show that the single target-specific mode-of-action paradigm is outdated; instead more models of modes-of-action are proposed and accepted today than there were decades ago when Bt crops were introduced. Consequently, an increasing number of non-target organisms are reported to be affected in many ways, outside of what used to be considered a limited range of target organisms. We call this the 'out-of-range paradigm'. We list 39 peer-reviewed publications that report significant adverse effects of Bt toxins on many 'out-of-range' species, including representatives from non-arthropod taxa, such as snails, crayfish, and bacteria. While this list of studies is not comprehensive (i.e. it is not an exhaustive review), it does illustrate the growing diversity of affected species and reported effects arising from Bt toxins that researchers have observed and reported, most of which cannot be detected in short-term acute direct toxicity tests that are applied in the first tier OECD testing protocols. In other words, these adverse effects of Bt toxins will be, and probably are already being, missed when using EFSA's approach of trying to reduce biology to chemistry.

We conclude that EFSA systematically excludes and ignores important exposure pathways and interactions of Bt toxins with whole communities of organisms in whole environmental compartments (e.g. aquatic ecosystems), and consequently it rejects scientific evidence of potential harm to non-target organisms if it does not confirm their expectations, and fails to recommend further studies when there is scientific uncertainty. Therefore, EFSAs risk assessment of Bt plants fails – by default (or maybe by design).

4.3 Assessment of health risks associated with the consumption of products derived from GE plants that are changed in their nutritional composition

Three GE crops with altered oil content have already been approved for import to the EU and use in food and feed. In future, other nutritionally altered GE crops – for example, with altered vitamin or mineral content – might be proposed for import or for cultivation, or might pose risks associated with the accidental spillage of crops granted authorisation for feed and food uses only.

Nutritionally altered GE crops pose challenges for risk assessment. Nutritional changes are complex and not limited to a single nutrient and their impacts may vary with dose and also depend on the

receiving population, which will include vulnerable persons. Due to this complexity, risk assessment and labelling are both challenging.

Nutritionally altered GE crops have been approved for use as food and feed within the EU without specific guidance for their risk assessment. This means that many important issues have not been considered adequately. Those issues include:

- the GE traits all affect multiple nutrients and the overall effect on health is poorly understood: health claims (of benefits) are not substantiated;
- because, unlike previous GE crops, nutritionally-altered GE crops are engineered to produce molecules that are biologically active in humans, there is an increase in the risk of adverse medical effects either from over exposure to the intended product or from unintended by-products whose hazard to health is unknown;
- gene-environment interactions will affect nutrient expression and the field trials conducted are inadequate to characterise the resulting variability in nutrient levels;
- there has been no full nutritional/food safety analysis (instead the focus is on comparing the main altered nutrient with standard dietary recommendations);
- potentially vulnerable subgroups need to be considered;
- impacts of food processing and storage need to be considered for all food types;
- use of the GE crop as feed can alter nutrient content of (unlabelled) meat and dairy products;
- food labelling proposals are inadequate to provide sufficient information for consumers; and
- post-market monitoring is inadequate to identify adverse health effects.

Other nutritionally altered crops may in future contain altered levels of vitamins and minerals, which will pose additional challenges for risk assessment.

No applications for commercial cultivation of nutritionally-altered GE crops have been made to date in the EU or Switzerland. However, there are many examples of unintended effects described in the published literature. These include:

- direct adverse effects on wildlife of consumption of altered nutrients;
- complex ecological effects associated with introducing new or enhanced levels of nutrients into ecosystems;
- increased attractiveness to pests and/or susceptibility to pathogens, associated with altering biochemical pathways in the plant; and
- adverse impacts on yield and agronomic properties.

In addition, contamination issues associated with nutrient-altered GE crops could be particularly significant: for example, with omega-3 altered GE oil seed rape being proposed for growing on an industrial scale for use as fish feed.

4.4 Assessment of health risks associated with the consumption of products derived from GE plants with a combination of traits

Most GE plants (events) allowed for import, processing and usage for food and feed into the EU, show a combination of several traits. These combinations can be derived from the stacking of plants (crossing of parental GE plants) as well as by co-transformation of single events. Most GE plants with stacked traits combine herbicide tolerance (HT) (also called herbicide resistance) and production of insecticidal toxins (IT) (also called insect resistance). The GE plants on the market

with trait combinations, especially those produced through stacking, are increasing and this trend is expected to continue in the future.

These combinations should always be addressed by the risk assessors. The harvests of HT plants regularly contain residues from spraying with the complementary herbicides such as glyphosate and others. In addition, one or several Bt toxins can be present. In regard to food safety, the combined presence of herbicide residues and insecticidal toxins (also in combination with specific plant constituents, e.g. with hormonal or immunogenic properties) have to be considered as stressors with potential additive, antagonistic or synergistic effects and interactions.

EU legal provisions such as Regulation (EC) No 1829/2003 (Recital 9) state that *"…any risks which they present for human and animal health and, as the case may be, for the environment…"* have to be avoided. Therefore, potential adverse effects that result from combinatorial exposures of various stressors need specification and their assessment needs priority. However, so far, the EU does not have a systematic and coherent approach to how health effects or environmental impacts stemming from such combinations of stressors should be assessed. In this report, the current gaps in risk assessments as performed by European Food Safety Authority (EFSA) are exemplified by two case studies.

We have analysed the concepts and methodologies for combined, cumulative and aggregated exposure to mixtures of stressors in GE plants with trait combinations. In addition, we discuss how other biologically active substances present in plants (e.g. oestrogens, allergens and anti-nutritional compounds in soybean) may interact with the trait-related characteristics and resulting stressors.

We have concluded that the health risk assessments as currently performed by EFSA for stacked GE plants are unacceptable. EFSA's approach does not take account of adverse health effects arising from GE plants, which could simultaneously introduce multiple potential stressors into our food chains. Our report shows that combinatorial effects (or potential mixed toxicity) emerging from simultaneous exposure to a fixed combination of potential stressors, emerging from GE plants at the stage of consumption, need to be assessed in far more detail.

We recommend that these plants should be tested following the whole mixture approach, considering them as "*insufficiently chemically defined to apply a component-based approach*" (EFSA, 2019c). For regulatory purposes, the plants should be considered as being equivalent to UVCB substances (substances of unknown or variable composition, complex reaction products or biological materials) as defined by the provisions of Regulation (EC) No 1907/2006 (REACH).

Currently, the most appropriate method to test these substances is life-time feeding studies with whole plant materials. This material should be relevant to the product consumed as food or feed, including the residues from spraying with complementary herbicides (with dosages that are in accordance with the conditions of commercial agricultural practices). To generate reliable data for products that are used daily in the food chain, the feeding studies will need to be long-term, including several generations.

In addition, in vitro testing systems and testing systems using non-vertebrates should also be required and developed further to establish risk-hypotheses and to reduce the overall number of animals needed for feeding studies. Further methodologies need to be developed for testing whole mixtures in addition to, or as reliable replacements for, animal feeding studies. More scientific studies should be initiated to better understand combinatorial, aggregated or cumulative exposure and effects from mixtures of GE plants in the diets of humans and animals.

As a next step, EFSA risk assessments and monitoring of mixtures of GE plants in diets that will lead to co-exposures of multiple potential stressors will need to fully assess the risks of combinatorial, aggregated and/or cumulative effects.

4.5 Assessment of environmental risks associated with GE crops that can persist and spontaneously propagate in the environment

If GE plants can persist and propagate in the environment and produce viable offspring, new challenges for risk assessment arise. For example, it is necessary to assess the extent to which the outcome of the risk assessment of the original event can be applied to subsequent generations, especially if those plants result in gene flow to and from wild relatives, as the effects of gene flow largely depend on interactions with the environment and the genetic characteristics of the plants.

Therefore risk assessors should assume that the characteristics of volunteer offspring and subsequent generational effects cannot be reliably predicted solely from the characteristics of the original event. New biological characteristics may be triggered in the offspring by their interactions with environmental conditions, making it difficult to reliably predict long term environmental impacts under changing environmental conditions, such as those caused by climate change.

Therefore, risk assessments of GE plants that can persist and propagate in the environment cannot be reduced to assessments of the specific traits and characteristics that are known at the initial stage of application, but need also to take into account effects that can emerge after some generations, in a broad range of environmental and genetic contexts and/or under stress conditions.

Furthermore, we have shown that exacerbating weed problems, displacement or even extinction of native plant species are not the only risks that might arise from persistent and self-propagating GE crops, as was suggested by EFSA in 2010. Much more weight needs to be given to the assessment of plants' interactions and biological signalling pathways and networks, such as those within the food web, with soil micro-organisms and insects such as pollinators and others. Those networks, for example, can be disturbed or disrupted by changes in the composition of volatile compounds or biochemical pathways and changes in nutritional quality.

In general, risk assessment of GE organisms that can persist and spontaneously propagate in the environment (within or beyond the production systems) poses some novel challenges to risk assessors and regulatory authorities, since the spatio-temporal dimension is a complex issue compared to GE plants only grown for one season. From our review we have concluded that risk assessments of GE organisms, which can persist and spontaneously propagate in the environment, need to deal with a degree of spatio-temporal complexity that can result in high levels of uncertainties. To deal with these problems, we recommend establishing 'cut-off criteria' in risk assessments that take into account the factual limits of knowledge. It is proposed to apply these criteria in a specific step within risk assessment called 'spatio-temporal controllability' that uses some well-defined biological characteristics to delineate some of the boundaries between known and unknowns considered to be crucial. Consequently, this additional step in risk assessments should enhance the robustness of risk assessments and substantially improve the reliability of decision-making about potential releases.

If it is known that GE organisms can escape 'spatio-temporal controllability', because they can propagate within natural populations, with no effective control of spread or persistence, then the authorisation process should not proceed and the release of the GE organism must not be allowed.

These criteria should not only be applied to applications for releases and cultivation but also to imports that are likely to cause spillage of viable kernels of relevant species (such as oilseed rape in the EU). In general, the release of GE plants should not be allowed if their persistence in the environment cannot be controlled in the spatio-temporal dimension.

4.6 Risk assessment of organisms derived from new genetic engineering technologies

GE organisms, in the form of plants, have been grown commercially in some countries, notably the Americas, since the mid-1990s. Current GE organisms have been developed using 'first generation' genetic engineering technologies. More recently, new applications of GE organisms and new modes of creating novel traits have been developed alongside new genetic engineering technologies. Grafting, cisgenesis and intragenesis, reverse breeding and RNA-directed DNA methylation (RdDM) either utilise GE organisms created using first generation techniques as an intermediary stage or can, in the case of agro-infiltration, unintentionally give rise to GE organisms. Most, if not all, of the principal concerns regarding first generation GE organisms apply to these new types of GE organisms and new genetic engineering techniques. Some novel types of GE organisms, e.g. RNAi-based GE plants present additional challenges for risk assessors, as do new genetic engineering techniques, such as genome editing.

<u>RNAi-based GE crops</u>

For RNAi-based GE crops, major uncertainties and knowledge gaps exist, resulting in open questions about how to assess the risks of RNAi-based GE crops to both the environment and the food chain. Despite the lack of EFSA guidance on the risk assessment of RNAi-based GE crops, three RNAi-based GE crops have been approved for food and feed use in the EU. This is not acceptable and RAGES strongly recommends that the issue of risk assessment guidance for GE organisms developed through new techniques, particularly those developed by genome editing, precedes any consideration of applications to cultivate or market.

<u>'Genome-editing'</u>

New techniques to create GE organisms have been developed in the past decade. In particular, the so-called 'genome editing' technologies have been much discussed. These include oligonucleotide-directed mutagenesis (ODM), Zinc-finger nuclease (ZFN), transcription activator-like effector nucleases (TALEN), meganucleases and CRISPR (Clustered regularly interspaced palindromic repeats) techniques: with CRISPR/Cas becoming the predominant 'genome editing' technology. Genome editing tools can also be applied to produce cisgenic, intragenic and transgenic organisms, applied to synthetic genomics and to induce RdDM.

'Genome editing' techniques can give rise to a broader spectrum of new genetic combinations and novel traits compared to the typical traits introduced by first generation GE organisms (predominantly herbicide tolerance, production of insecticidal proteins and combinations thereof).

However, 'genome editing' is limited in its applications when it comes to editing of polygenic traits. Whilst several sites in the genome can be targeted at once, these are edited outside of their genetic and epigenetic regulation. Many characteristics required by farmers and/or consumers (e.g. drought tolerance in plants) are controlled by 'complex traits'. Modern conventional breeding techniques such as genomic selection and marker assisted selection are, in general, more suited to breeding complex traits. One principal reason is that, with conventional breeding the whole genome is encompassed, so that genetic and epigenetic regulation of genes remains intact. Conventional

breeding has had, and will undoubtedly continue to have, success in breeding varieties with traits such as enhanced drought and/or flood tolerance.

EU regulation covers genome editing

‘Genome editing’ technologies involve the direct modification of genomes. That means that changes in the genome are achieved by directly introducing either genetic material or material that enacts a change to genetic material in cells, with the material produced, or at least handled in the laboratory, by humans. This concept of direct modification of genomic material is important as it underlies the concept and definition of both a GE organism (GMOs) in the EU and a living modified organism (LMO) in the UN Cartagena Protocol on Biosafety.

Broadly, more recent genetic engineering techniques can be grouped into three groups:

1. those giving rise to novel types of GE organisms (synthetic genomics, RNAi-based crops, cisgenesis and intragenesis);
2. infrequent applications of GE organisms in plants (grafting; agro-infiltration; reverse breeding) and
3. new techniques of producing GE organisms (RdDM and genome editing techniques: ZFN, ODM, CRISPR, TALEN, meganucleases).

Unintended effects

As with plants developed through first generation genetic engineering technologies, both intended and unintended changes can be important in terms of gene products such as proteins, metabolism and resulting biological effects. Thus, it is possible, even likely, that, like first generation techniques of genetic engineering, genome editing techniques will give rise to plants displaying unexpected and unpredictable effects with implications for food, feed and environmental safety. Although genome editing techniques are often described as ‘precise', in reality there is substantial potential for unforeseen genomic interactions, genomic irregularities and unintended biochemical alterations. These can produce unexpected effects in the resultant GE organism.

Unintended effects associated specifically with genome editing fall into two main categories:

- off-target effects where the nuclease unintentionally alters DNA at a site in addition to the target site;
- unintended on-target effects, where the intended change generates further alterations, e.g. to genomic regulation.

Farm animals

Currently, there are no commercial GE farm animals in Europe, and the only GE animal approved for food use is limited to a GE salmon in Canada and the USA. The production of GE animals is thought to have been limited by difficulties with first generation genetic modification techniques. In contrast, CRISPR is claimed to have high efficiencies in animals, meaning that there may soon be applications to market genome-edited farm animals as food. But besides risk-related issues, ethical and welfare concerns of genome-edited animals are pressing and largely similar to those that have been raised for transgenic animals and/or cloning.

Gene Drives

Gene drives are genetic elements that do not follow the Mendelian pattern of inheritance as they increase the probability that a specific genetic condition is being transmitted to the next generation above the normal 50% for sexual reproduction. With gene drives, contrary to most other applications of genetic engineering, the GE organisms are not intended to be contained within the

laboratory or restricted to a single generation of domesticated plants, grown within fields. They are intended to genetically engineer wild (uncultivated) populations of animals and plants. In this context, new layers of risk-related issues emerge, including a lack of spatio-temporal control and disruptive processes that can affect whole species and/or associated ecosystems. Gene drives, no matter whether they are supposed to replace or suppress a population, can give rise to GE populations that persist in the environment with little or no opportunity for recall. If persistence of GE organisms goes along with lack of spatio-temporal control, it becomes difficult or impossible to predict either their short-term or long-term ecological impacts. There is a broad range of further negative or adverse impacts that require consideration by risk assessors and risk managers, such as spontaneous transboundary movements, introgression into organic production systems in agriculture, and socio-ecological and ethical considerations. As a consequence, there are many serious and valid concerns regarding the uncontrolled spread of organisms with gene drive systems. It is not clear how the approval of local communities could be sought (as required under the Conventional for Biological Diversity), as at present there is no mechanism for societal consultation on GE organisms in the EU. Application of the Precautionary Principle, as enshrined in EU law, would in any case preclude the release of GE organisms as part of a gene drive system.

Risk assessment for organisms developed through genome editing techniques

Just like first generation techniques, new genetic engineering techniques can produce unexpected and unpredictable effects in the resultant GE organisms, even if additional genes are not inserted or any inserted genes are subsequently removed prior to commercialisation. Therefore, it is important that any applications for cultivation (including field trials) and marketing of GE organisms produced by these techniques undergo full environmental and health risk assessments. The current risk assessment guidance in the EU would need to be expanded in order to assess the additional unintended effects that genome editing can cause. For example, the molecular characterisation element of the risk assessment will need to be expanded to include analysis for unintended changes at the genomic level, including off-target effects, unintended on-target effects and effects on genomic regulation.

There are several techniques that can be used to detect and assess any unintended effects generated by the genome editing process. These could also be used to improve the risk assessment of GE organisms created by first generation techniques. These are collectively summarized as ‘omics’ approaches and include analysis of the RNA profile (transcriptomics), the protein profile (proteomics) and the metabolite profile (metabolomics). Metabolic profiling characterizes the current status of all molecules involved in the metabolism using methods combining chromatography and spectrometry.

The risk assessment will need to consider a broader range of traits conferred by the genetic engineering process, for some of which there may be a lack of experience. It will need to consider direct and indirect implications for agricultural practices and ecological impacts caused by, for example, any changed animal diets. Genome-edited GE plants should also be analysed in regard to the composition of their microbiome as the microorganisms colonizing the surfaces and inner tissues of plants play, for example, an important role for functional traits of the plant such as crop yield and nutrient quality as well as for soil fertility and functioning of the ecosystems.

Detectability of GE organisms developed with new techniques

As with current GE organisms, labelling of GE organisms created by genome editing will be necessary to facilitate consumers' choices and to protect agricultural systems that exclude GE organisms, e.g. organic agriculture. GE organisms developed by 'genome editing' are detectable, provided prior information is available regarding the intended genomic changes. It is evident that advances in detection technologies are needed, not only for genome-edited organisms, but for other new genetic engineering techniques such as RdDM as well. Therefore, there needs to be the political will and investment to develop suitable and adequate detection technologies. Regulatory requirements of traceability and labelling would be likely to spur research into developing new detection technologies.

5. Conclusions and recommendations

Existing gene combinations and biological characteristics in living organisms are derived from three to four billion years of evolution. For a few thousand years humans have used existing biodiversity to breed plants and animals for food production by selection and subsequent crossing. In more recent history, we have also used techniques such as mutagenesis to enhance genetic diversity within shorter periods of time for further crossing and selection.

These methods of conventional breeding have all been based on natural diversity and evolutionary mechanisms. They are profoundly different from those of genetic engineering. By directly introducing biological material, such as DNA prepared outside the organism, genetic engineering techniques allow mechanisms of natural heredity and gene regulation to be by-passed. Thereby allowing the introduction of biological characteristics that are not derived from evolutionary mechanisms and existing biodiversity.

The application of these techniques is not restricted to altering the genomes of domesticated plants and animals for food production; they could also be used to engineer wild populations. This is especially relevant for new methods of genetic engineering ('genome editing'). Especially under these conditions, spatio-temporal complexity causes a high level of uncertainty and also profound ignorance. The biological consequences emerging from a "crack in creation" (Doudna & Sternberg, 2017) and their long-term impacts can not yet be reliably predicted or assessed. Remarkably, the inventors of the CRISPR technology themselves are warning about "how radical the implications of gene editing are for our species and our planet." (Doudna & Sternberg, p. 243)

Many stakeholders in the field, company representatives as well as academics, are trying to create the impression that current risk assessment methods are sufficient to identify and control the risks, but this is misleading.

Those stakeholders claim that so far no major or acute damage has been observed, and therefore claim the overall safety of those plants which have been approved and cultivated. However, such statements give a misleading impression and are not scientifically robust:

- There are many issues being overlooked and not taken into account by current EU risk assessments, e.g. interactions of the additionally inserted genes with the plants' genomes, changes in the associated microbiomes, reactions to stress conditions and subsequent generational effects. Although these issues might not be considered as damage per se, they show that overall safety of these plants remains subject to major uncertainties.

- There are crucial aspects such as combined effects and mixed toxicity that are intentionally excluded from EFSA's risk assessments.

- At the same time, more and more complex biological mechanisms are being discovered and described, which govern gene regulation, signalling pathways, ecological networks and environmental interactions, but so far they are completely outside the scope of EFSA's existing risk assessment guidelines.

We are aware that the EU Commission has previously given several statements claiming in a rather 'populist' way that there would be no specific risks associated with GE plants, such as:

> *"The main conclusion to be drawn from the efforts of more than 130 research projects, covering a period of more than 25 years of research, and involving more than 500 independent research groups, is that biotechnology, and in particular GMOs, are not per se more risky than e.g. conventional plant breeding technologies."*

This statement is taken from a titled called "*A decade of EU-funded GMO research (2001 – 2010)*" (EU Commission, 2010) and is frequently quoted by stakeholders being interested in development and marketing of GE organisms. The EU Commission report mostly provides technical details relating to the development of GE organisms and their potential applications. However, it does not deal with risk assessment in regard to health and the environment as discussed and elaborated within the RAGES project.

Moreover, the report was published in 2010 and is mostly outdated. It does not deal with current agricultural practices, 'stacked events' or more recent publications and new issues that have arisen over the last ten years; these were the issues discussed and considered in the RAGES project.

The outcome of RAGES shows how risk assessors in the EU and Switzerland are failing to deal with the real and more recent problems. In many cases, they are following a 'don´t look & don´t find' approach, which does not take into account the limits of knowledge, and does not identify crucially important uncertainties or knowledge gaps. Risk assessors are following a reductionistic, restrictive evidential approach, which largely ignores the complexity of life forms and their interactions with their environments as well as evolutionary principles. Therefore, we conclude that current risk assessment of key safety issues, as performed by EFSA and the Swiss authorities, is failing by design.

The findings of the RAGES project show that the Precautionary Principle needs to be applied far more consistently and comprehensively. While each sub-report presents a list of specific recommendations, there are several overarching implications of those findings:

- More risk research needs to be carried out independently of stakeholders interested in the development and commercialisation of GE organisms.

- Risk assessment policies have to be developed to address gaps in current knowledge and inadequacies in prevailing approaches to assessing risks. Policies also need to be developed and implemented to engage with new findings in biology, and to develop new investigative methodologies as well as improve guidance for risk assessments.

- Spatio-temporal control is key for the implementation of precaution if GE organisms are released. Without such possibilities for control, effective measures cannot be taken if adverse effects and damage to the environment occur. No releases of GE organisms can be allowed if they cannot be prevented from persisting and propagating in the environment. Applications with inadequate spatio-temporal control include gene drive organisms.

- Wild/natural populations should be strictly protected against gene flow from GE organisms.

- Field trials have to be conducted under conditions which represent the real agronomic practices under which the plants are expected to be grown commercially.

- The responses of the plants to changes in environmental conditions e.g., climate change, have to be taken into account.

- Much more weight should be given by EFSA to assessing cumulative and combinatorial effects that can arise from events that transmit more than one trait or by mixing products of several events in one diet.

- Combinatorial and accumulated effects also have to be assessed if plants with more than one trait are cultivated, or if several events are grown in the same region.

- New mechanisms for monitoring post-release impacts need to be developed; the can help to close gaps in current knowledge. Research is needed to provide more information about more subtle long term effects, even in cases where these were not already identified as manifest adverse effects during the process of risk assessment.

- Organisms that result from processes of ‘genome editing’ should have to undergo an approval process and be labelled in accordance with EU GMO regulation. Specific guidance is needed to define detailed assessment of their risks.

As long as the gaps in EFSA’s risk assessments are not closed, the safety of the GE organisms cannot be ensured and market approvals should not be granted. Since in the past, risk managers (e.g. the European Commission and the Swiss Authorities) were not able or willing to face these challenges, it is important and urgent to start a process for improving the scope and rigour of EFSA’s risk assessment, and to ensure that policy outcomes fully comply with legislative requirements and public expectations.

Projects such as RAGES and ongoing research show a widening gap between EFSA’s current risk assessment practices and the actual complexity of relevant issues. Bringing more light to these issues and exposing the gaps in scientific knowledge and official practices may encourage political decision-makers to give proper weight to the protection of public and environmental health as required by EU legislative statutes.

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Annexes

Annex I:

The results of RAGES are published within six detailed subreports:

- Assessment of health risks associated with the consumption of products derived from herbicide tolerant GE plants;
- Assessment of environmental risks associated with the cultivation of insecticidal Bt crops;
- Assessment of health risks associated with the consumption of products derived from GE plants with altered nutritional composition;
- Assessment of health risks associated with the consumption of products derived from GE plants with a combination of traits;
- Assessment of environmental risks from the persistence, self-propagation and uncontrolled spread of GE plants; and
- Risk assessment of GE organisms derived from new genetic engineering technologies.

Annex II:

Table 1-4: Overview on cross cutting gaps and deficiencies in current risk assessment as currently performed in the EU and Switzerland