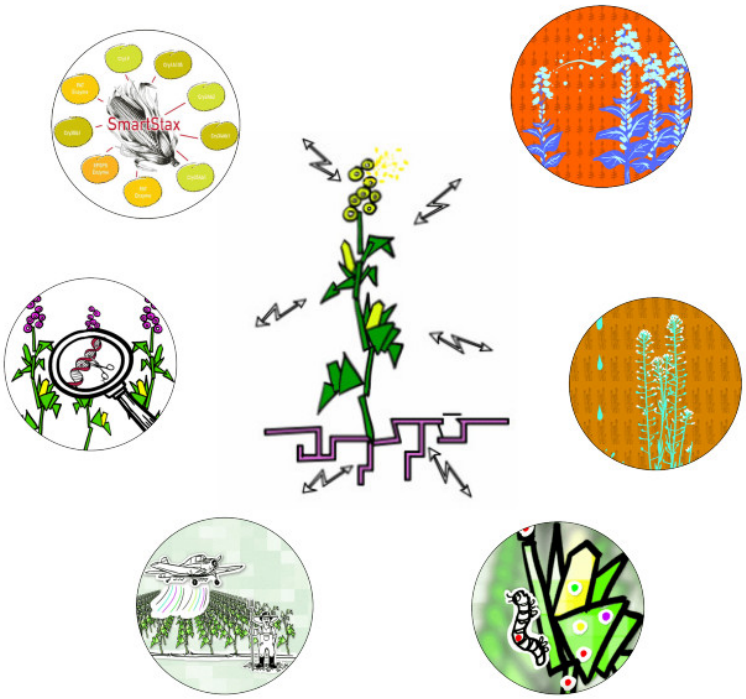
RAGES

RISK ASSESSMENT OF GENETICALLY ENGINEERED ORGANISMS IN THE EU AND SWITZERLAND

Tabled overview on cross cutting gaps and deficiencies in current risk assessment as currently performed in the EU and Switzerland

Annex II to the overall report

January 2020



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STIFTUNG MERCATOR SCHWEIZ

Editorial:

Tabled overview on cross cutting gaps and deficiencies in current risk assessment as currently performed in the EU and Switzerland, Annex II to the overall report, www.testbiotech.org/projekt_rages

Published by ENSSER (https://ensser.org), GeneWatch UK (www.genewatch.org) and Testbiotech (www.testbiotech.org).

Figures: Testbiotech, Timo Zett, Claudia Radig-Willy

Funded by Stiftung Mercator Schweiz

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Table 1: the molecular level

Issue	Problem & Relevance	What should be done
Publication of data on DNA sequences that were changed or inserted.	The DNA sequences are highly relevant for risk assessment: DNA sequences can have direct implications for the biological quality of the *intended gene products*, such as Bt toxins: These toxins as expressed in the plants are not produced from a DNA that is identical to the native variants found in soil bacteria. In most cases, the DNA is truncated or changed in its structure to render it more efficient. These changes in the structure of the DNA are highly relevant for risk assessment for environmental impacts as well as food safety. Furthermore, the DNA sequences of the gene constructs and the structure of the DNA at the site of insertion are also very relevant for risk assessment of *unintended gene products.* Currently, EFSA does not adequately assess these gene products. For example, gene products that are not translated into proteins are completely ignored. If the relevant DNA sequences are not disclosed, the public does not have access to information relevant for risk assessment.	All data about site of insertion, changes in the plants DNA and the inserted gene constructs should be made publically accessible for independent experts.
Publication of full data on intended gene products that are produced in the plants.	In many cases, biologically active compounds such as Bt proteins are produced in the plants, which have natural counterparts, but are significantly changed in their structure. In other cases, biological active compounds are produced in the plants, that do not have natural counterparts. In both cases, it is important that independent experts have access to the full data about the composition and structure of these compounds, to improve the overall risk assessment.	Full data about the composition and structure of the intended gene products should be made publically accessible so they can be evaluated by independent experts.
All gene products from all new open reading frames should be identified	For example, miRNA is known to persist to some extent after ingestion and environmental degradation and could interfere with gene regulations across the kingdoms of living organisms.	All relevant data should be required from the applicant and made publically accessible so that independent experts can analyse the information.
Reliable methods for measuring expression of newly inserted genes should be applied	The methods used to determine the amount of Bt toxins and other gene products are known to be dependent on the specific protocols used. The data from a particular method are not sufficiently reliable without further evaluation by independent labs. Without fully evaluated test methods to identify and measure the expression and the concentration of the Bt toxins, EFSA's risk assessment suffers from	Fully documented test methods should be required, and evaluated by independent laboratories, and made available for analysis by independent experts.

Issue	Problem & Relevance	What should be done
	substantial methodological gaps.	
Impact of the genetic background	In interaction with changes in the genetic background, the additionally introduced genetic elements can show unexpected changes in expression.	EFSA should require data on gene expression of several varieties (for example the reference lines also used in other field trials), which are grown in test fields and in parallel. Companies should be obliged to report relevant data over all the years of all varieties being cultivated.
Genome x environmental interactions	Under stress conditions, genetically engineered organisms can exhibit characteristics not observed in the laboratory / normal field conditions such as changes in gene expression and plant metabolism.	Plants and seeds should be tested under controlled conditions to a wide range of defined (biotic and abiotic) stressors. Omics-data such as transcriptomics, proteomics and metabolomics should be requested. Those data should be used to assess the reaction of the plants on molecular level.

Table 2: plant composition and composition and agronomic/phenotypic characteristics

Issue	Problem & Relevance	What should be done
Step by step procedures are important before field trials are started.	It is assumed by EU and Swiss regulators that a step-by-step approach has to be established before any genetically engineered organisms are released into the environment. It is important, to define all the steps properly, to avoid unnecessary risks for the environment.	A defined set of molecular level data of the plants has to be obtained and reported, in tests under well-defined conditions, in both laboratories, climate chambers and greenhouses. Those data shall be made delivered to the authorities before any field trials are started. That information should include data on gene expression, changes in composition, phenotypical characteristics and interactions with the associated microbiomes.
Design of field trials	In most cases, field trials are only performed for one year and only in a few specific geographic regions. Furthermore, important comparators such as several varieties inheriting the same event or, in the case of stacked events, the parental plants, are often not grown in parallel in the field trials. On the other hand, reference lines are planted in parallel that - in many cases - are not suitable comparators to assess the differences between the genetically engineered plants and their counterparts.	Field trials should be performed over several years and in all relevant regions where these plants are supposed to be grown, taking also into account the regional agricultural practices and varieties. However, before starting any field trials, much more data should be produced under defined conditions, such as in the lab, climate chamber and green house, in an well organised step by step procedure (see above). Additional comparators should be grown in parallel, such as several varieties inheriting the same event and the parental plants in the case of stacked events. Further, the environmental conditions that occur during field trials should be documented in detail.
Changes in plants' composition and their agronomic and phenotypical	In many cases, many significant changes in the plants' composition and/or and their agronomic and phenotypical characteristics are reported in the data for application to EFSA.	A larger overall number of significant differences, or such effects that are observed consistently within all field sites, should trigger further investigations, even if, taken as isolated

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characteristics	In most cases, EFSA deals with each of those significant differences separately and in isolation. Also in cases where the data show that e.g. half of the parameters analysed were significantly changed, EFSA nonetheless assumes that the genetically engineered plants are not different from their comparators. As long as EFSA sees no evidence that the single changes are posing specific health risk, data that show significant differences between the genetically engineered plants and their comparators are mostly ignored. Further, under food and feed risk assessment, data which are derived from parts of the plant that are not meant for import, in most cases are not assessed in detail.	data, those effects might not raise safety concerns. Further studies should be required such as • data from omics (including proteomics, transcriptomics and metabolomics), • data representing more extreme environmental conditions such as those caused by climate change, • data representing more areas of commercial soy cultivation, • more data on stress reactions under controlled conditions and • more criteria to be tested, including all parts of the plants.
Genome x environment interaction	Under stress conditions, genetically engineered organisms can exhibit characteristics not observed in the laboratory or greenhouse. Plants might become more susceptible to diseases and pests or more persistent and invasive, or changes in metabolism and gene expression might occur that can cause adverse effects.	As a first step, defined sets of data about the molecular level of plants have to be produced and reported under defined conditions (see table 1). If field trials are conducted, the dataset from the first step should be used to stipulate the design of subsequent field trials, and taken into account when assessing outcomes. Data requirements should ensure that the plants are grown under a sufficiently wide range of environmental conditions, taking into account extreme conditions such as occurring under climate change.
All relevant biological active compounds should be tested.	In compositional analysis, often only some but not all biologically active compounds are tested and assessed. For example in soybeans, a large part of the known substances are effectively ignored.	Depending on the particular plant species and variety, and intended areas of cultivation, a wider range of biological compounds should be measured by the applicant, than currently required.

Table 3: Toxicology and allergenicity

Issue	Problem & Relevance	What should be done
The establishment of a step-by-step procedure, including *in vitro* studies.	So far, the only toxicological studies required are feeding studies with the isolated proteins and 90 day feeding studies with whole food. There are other, more targeted systems that should be developed further. *In vitro* systems such as cell cultures or isolated tissues should be used as a starting point before any feeding studies are conducted. Those systems can help risk assessors to develop hypotheses that later could and should	Risk assessors should require data from targeted i*n vitro* studies, which should be performed before any feeding studies are undertaken. Further, long term feeding studies over several generations are needed substantially to reduce uncertainties substantially. These would also bring new information, so far unprecedented.

Issue	Problem & Relevance	What should be done
	be tested in feeding studies.	
Assessment of effects on immune and reproductive systems	Tests on the immune system (allergenicity, adjuvantic effects or other immune reactions) as well as the reproductive/hormonal system are not currently required, despite the view of experts that they should be deemed relevant and important.	As adequate *in vitro* methods are not available, feeding trials focussed on those endpoints should be required. Additional data should be obtained from animal feeding studies under realistic commercial conditions.
Impact onto the gut microbiome during consumption, digestion and metabolism	Although it is known that the intestinal microbiome has significant impacts on the health status of the consumers, no data are required on the impact of the consumption of genetically engineered plants.	EFSA should require data on changes in the microbiome from animals fed with genetically engineered food & feed.
The use of material that was produced under practical conditions	Especially with herbicide resistant plant, tests should be conducted on samples produced under realistic commercial conditions, including exposure to high doses of herbicides and several applications. If those conditions are not met, the results are not reliably relevant.	Materials used for empirical studies should be produced under conditions that match realistic commercial conditions in the countries and regions of cultivation.
The use of material for feeding studies that is not contaminated with other GMOs or pesticides.	Currently EFSA accepts data from feeding studies even if the diets used for comparison were contaminated with, or produced from, other genetically engineered varieties. Consequently, relevant adverse effects might not be identified.	The diets used in the control groups of animal feeding studies should not contain any GMOs. The diets of the test groups should include the variety being tested, and also tested again with each of the other GM varieties with which it could be mixed in commercial practice. Further, all diets should be fully assessed in relation to likely the residues from spraying with pesticides, herbicides, fungicides that are likely to be used in commercial cultivation, as well as other ingredients that could adversely impact outcomes.
Assessment of combinatorial or cumulative effects of mixtures in the diet	In many cases, a specific pattern of residues from spraying due to usage of several complementary herbicides can be expected in combination with a several insecticidal proteins as produced in the plants. Samples with those combinations of compounds should be tested in regard to mixed toxicty. Those mixtures will occur in stacked events as well as in diets that mix several genetically engineered plants.	Where specific patterns of residues from sprays and/or other contaminants and stressors are anticipated to be present in specific products or diets, those residues and stressors should be tested and assessed in combinations.
Monitoring of the effects of genetically engineered plants used in animal feed and agriculture	In the EU, millions of tonnes of imported genetically engineered soybeans are fed to animals but no monitoring of specific health effects is required or performed.	Risk managers should ensure that effective monitoring of adverse health effects of animals fed with genetically engineered plants is established, and the resultant data evaluated by EFSA and made publically available.

Table 4: Environmental risks

Issue	Problem & Relevance	What should be done
Collection of detailed data on the receiving environment	So far, data from the receiving environments concerning the various regional conditions and the abundance of protected and/or endemic species and their susceptibility, the food web, the regional geoclimatic conditions and the general vulnerability of the agro-ecosystems are largely missing. However these data are needed to conduct sound environmental risk assessments. Also modelling of exposure and the selection of key organisms for detailed investigations is depending on those data.	All regions where genetically engineered plants are cultivated have to be assessed in regard to their specific conditions and the abundance of endemic or protected species. Empirical data on the susceptibility of organisms, which are exposed to stressors such as Bt toxins, have to be provided and published. Methods should be developed that allow risk assessors to judge the overall vulnerability of the receiving environments and agro-ecological systems.
Assessment of effects on wildlife species and the whole food web	Data from wildlife species such as birds and wildlife mammals are not currently required at all. Only small selections of other parts of the relevant food webs (in the soil, in aquatic systems, above soil) are considered by EFSA. Cumulative effects of plants ingredients such as Bt toxins are largely ignored.	The risk assessors should establish an overview on relevant foodwebs in the various regions before starting risk assessments of specific events.
Taking into account communication networks (“the interconnected environment”)	For example, communications and interactions with pollinators or the defense mechanisms of plants can be impacted by the introduction of new metabolic pathways. But volatile compounds and other constituents of the secondary metabolism or biological active compounds which are involved in these communication pathways are not yet part of EFSA’s risk assessments.	The plants and their associated communication networks should be seen as an integrated system that have to be subjected to risk assessments by EFSA. The EFSA risk assessors should properly identify and characterise the limits of their knowledge and develop adequate methodology to reduce key uncertainties.
Impact on associated microbiomes (“the associated environment”)	The well-established concept of the holobiont takes into account the associated microbiome (such as intestinal flora, mycorrhiza) which is decisive for the overall biological characteristics of the organisms and its effect on the environment.	The plants and its associated micro-flora should be seen as an integrated system that has to be subjected to risk assessment. The EFSA risk assessors should properly identify and characterise the limits of their knowledge and develop adequate methodology to reduce key uncertainties.
Life cycle of the additional proteins	Bt proteins, for example, are produced throughout the whole life cycle of the plants. The environment can be exposed via various pathways such as roots, pollen and other parts of the plants. The source of the proteins might be cultivation, harvest, processing or discharge from animals. To assess the impact of these newly introduced proteins, their life cycle, potential cumulative effects and their degradation products in various terrestrial and aquatic systems have to be studied and assessed.	Depending on the trait and the intended purpose, data on life cycle and degradation products of newly introduced proteins should be required and published.
Genome x environment interaction and	Under stress conditions, genetically engineered organisms can exhibit characteristics (changes in gene regulation) that are not observed in the	The changes in gene expression of the plants in reaction to changed environmental conditions should always be one of the starting points in a

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epigenetic effects ("the reaction to the environment")	lab or greenhouse. Gene regulation can impact the biological characteristics of organisms / populations without changes in the DNA. These effects can for example affect the plants characteristics under changed environmental conditions.	risk assessment. The EFSA risk assessors should require data from a broad range of environmental conditions and stressors, also taking into account continuing climate change.
Combinatorial effects of stacked events	Cumulative effects from stacking of several traits have to be assessed in regard to additive and/or synergistic effects. In this context, for example the combinatorial effects between Bt plants and/or HR plants are relevant.	Potential combinatorial effects should be tested in the laboratory using well-established model organisms. In addition, empirical data should be required for protected and/or endemic species.
Combined/ cumulated effects from parallel cultivation of several GE plants	Cumulative effects from combined cultivation are required to be assessed according to Directive 2001/18. In this context, for example the combinatorial effects between Bt plants and/or HR plants are relevant.	The EFSA risk assessors should properly describe limits of their current knowledge and develop adequate methodology to address cumulative effects. As a first step, potential combinatorial effects should be tested in the laboratory using *in vitro* systems and well established model organisms.
Long term and large scale effects	Effects occurring on the level of large populations after longer periods of time can be significantly different from those observed in the lab or in field trials. This can, for example, concern biodiversity, soil, the food web and also long term effects in the food chain.	EFSA risk assessors should properly describe limits of knowledge and develop adequate methodology to address long term effects. Risk manager should ensure that effective and specific monitoring is established in all regions were genetically engineered organisms are released.
Subsequent generational effects	If genetically engineered organisms persist in the environment and propagate, or if gene flow to other cultivated crops occurs, subsequent generations might show unexpected characteristics that cannot be predicted just by assessing the original events on its own. By interacting with changes in the genetic background, the artificially introduced genetic elements could show unexpected characteristics. These effects will be especially relevant if the event is crossed with new varieties as well as if gene flow occurs to wild relatives.	Spatio-temporal controllability should become a cut-off criterion in EFSA's risk assessments. In any case, if spontaneous reproduction and / or gene flow cannot be ruled out, EFSA should require empirical data on subsequent generational effects. The risk manager has to make sure that no uncontrolled spread of the genetically engineered organisms into the environment can occur.
Effects of hybridisation with wild relatives	If gene flow occurs from genetically engineered plants to wild populations and/or relatives, the biological characteristics (such as fitness) of the offspring can be largely different from those of the parental organisms.	Spatio-temporal controllability should be a cut-off criterion in EFSA's risk assessments. Assessments of potential gene flows to wild populations should be included in EFSA's revised assessment methodology. The risk manager has to make sure that no uncontrolled spread of the genetically engineered organisms into the environment can occur.