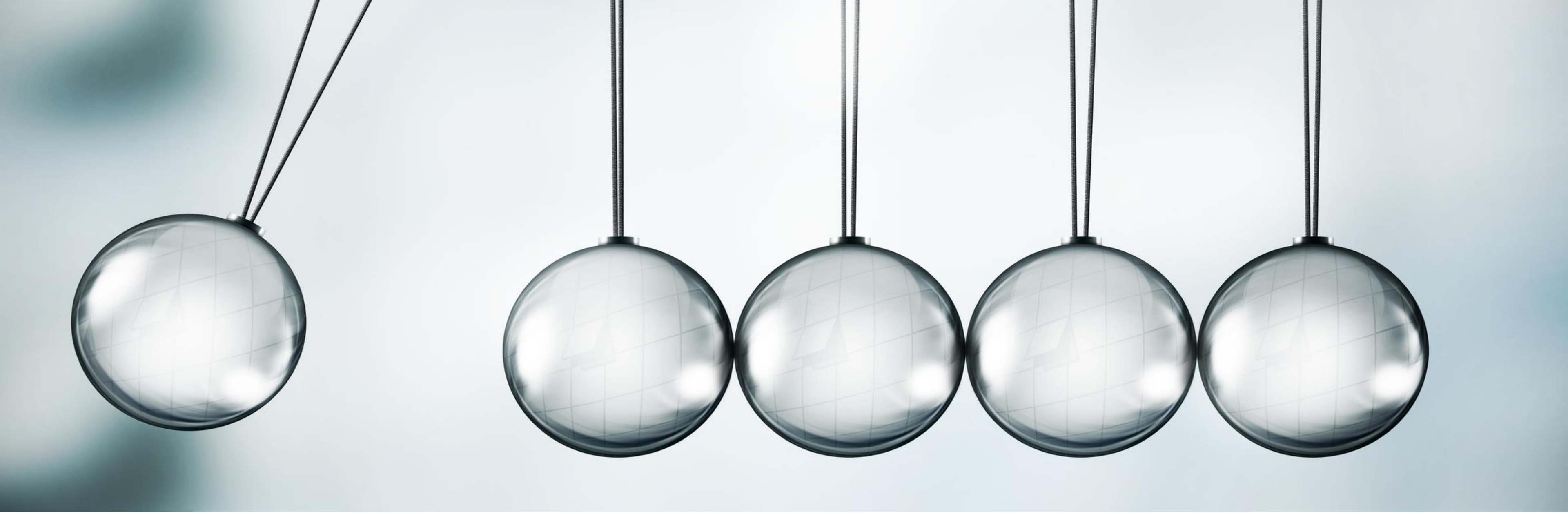

BIOSAFETY CONSIDERATIONS FOR PLANTS DEVELOPED BY GENOME EDITING AND OTHER NEW GENETIC MODIFICATION TECHNIQUES (nGMS)

MICHAEL ECKERSTORFER

PERSPEKTIVEN FÜR UMWELT & GESELLSCHAFT umweltbundesamt

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POLICY AND PRACTICE REVIEWS
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An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs)

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Plants Developed by New Genetic Modification Techniques—Comparison of Existing Regulatory Frameworks in the EU and Non-EU Countries

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- Scope of Techniques and Terminology
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- Regulatory issues
  - Coverage by existing regulatory frameworks for GMOs (Regulatory trigger)
  - Future regulatory development (amendments existing regulations / new regulations)
- Enforcement issues
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QUESTIONS CONCERNING nGMs / nGM-plants addressed in a study commissioned by BfN

Biosafety Considerations for nGM Plants – a perspective based on an European (EU) background

Comparison of existing regulatory frameworks for nGM plants (EU and non-EU countries)

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- Survey of scientific literature
  - Which nGMs? (Techniques, Objectives)
  - Plant applications? (Crops, traits, use in agricult.)
- Risk assessment considerations
  - nGM characteristics relevant for RA
- Considerations for a case-specific framing of the RA
  - Molecular characterisation

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- Comparison of existing regulatory frameworks
  - Similarities & differences
- Pros & Cons of respective regulatory triggers
  - Product- or process-oriented trigger definitions
- (Possible) regulatory approaches
  - Options for regulation of nGM products

nGMs: GM-TECHNOLOGY & BEYOND …

1. **Genome Editing (GEd)** applications directed to introduce genomic modifications in a targeted manner, e.g. by means of site directed nucleases (SDNs) or ODM
   - SDN-1 / SDN-2 / SDN-3 / ODM / base editing
2. **Cisgenesis or Intragenesis** applications, where only genetic material derived from the parental or sexually compatible species is used for genetic modification
3. **GM-modifications** present only **transiently** or **in particular plant parts**
   - e.g. agroinfiltration, transgrafting (GM rootstocks)
4. **Applications** where the **GM-modification(s)** are **only present in intermediate breeding steps** and supposed to be absent from the final product
   - e.g. reverse breeding, haploid induction, accelerated breeding/early flowering, etc.
5. **Applications** directed **to modify the epigenetic regulation** in plants and their offspring rather than the DNA sequence of their genomes
   - e.g. RNA-directed DNA Methylation (RdDM), SD-epigenetic effectors

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nGMs / Applications	Genome editing					RdDM	CG	IG	TG*	nGMs to support breeding		Total:
	CRISPR*	TALEN	ZFN	MN	ODM					AI	HI	
JAN. 2011–DEC. 2015												
Total number	**n.a.**	**10**	**17**	**5**	**1**	**6**	**7**	**4**	**n.a.**	**14**	**9**	(73)
JAN. 2016–JUNE 2017*												
Total number (172)	**114**	**8**	**7**	**1**	**1**	**1**	**2**	**4**	**23**	**4**	**7**	172
SDN–1	99	5	4	–	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
SDN–2	5	–	–	–	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
SDN–3	4	3	3	1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Base editing	4	–	–	–	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Other types of genome editing	2	–	–	–	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
OBJECTIVE OF APPLICATIONS (JAN. 2016–JUNE 2017)												
Method development	72	1	2	1	–	–	1	1	6	–	3	88
Basic research	22	1	2	–	–	–	–	–	7	4	1	37
Applied development	20	6	3	–	1	1	1	3	10	–	2	47

SDN, site-directed nuclease; CRISPR, CRISPR (Clustered regularly interspaced short palindromic repeat)-directed nuclease; TALEN, Transcription activator-like effector nuclease; ZFN, Zinc-Finger-directed nuclease; MN, Meganucleases; ODM, Oligonucleotide-directed mutagenesis; RdDM, RNA dependent DNA methylation; CG, Cisgenesis; IG, Intragenesis; TG, Transgrafting; AI, Agro-infiltration; HI, Haploid induction; Other types of genome editing: different variants of CRISPR-based genome editing, including use of nickases; n.a.: not applicable.

**For the use of CRISPR-based systems for genome editing and transgrafting literature was only screened for the time period Jan. 2016-June 2017.*

Bold values indicate total numbers of publications for individual nGMs for the indicated time periods.

RESULTS OF LITERATURE SURVEY /1

- Continous interest in all nGMs (2011-2017: 321 publications, 245 + 76/CRISPR Hilscher et al. (2017) Biotechnol. J. 12, 600173)
  - Clear focus on **GEd**(**75%** of total publications)
  - Wide range of plant species:
    model species, important crop species (grain and oilseed crops, vegetables and spices, perennial plants (fruit and forest trees) and mosses
- Focus of recent work (Jan 2016 - June 2017, 172 publications)
  - Strong focus on **GEd by SDNs** (**75 %** of total publications)
  - Most genome editing of **SDN-1** type (**83 %** of GEd publications)
    Knock-out of endogenous genes (basic research, applied development)
- Strong recent focus on CRISPR-applications (**88 %** of GEd publications)
  - CRISPR-**technology development** (**>60 %** of CRISPR publications**)**
- Objective for use (Jan 2016 - June 2017)
  - Method development (**51 %**, CRISPR, haploid induction),
  - Basic research (**22 %**, CRISPR, agroinfiltration),
  - Applied development (**27 %**, TALEN, ODM, cis/intra, transgrafting)

RESULTS OF LITERATURE SURVEY /2

- Method development aims (General)
  - Increased efficacy and speed of methods
  - Increased range of plant species to be modified
  - Products without integrated transgenes

- Method development aims (Genome editing, CRISPR)
  - Enhanced specificity and targeting
  - Other CRISPR-nucleases than Cas9 (e.g. Cpf1)
  - deadCas9-fusion enzymes for nicking, base editing or transcriptional/epigenetic regulation
  - Multiplexed applications (multiple guide RNAs) – e.g. for metabolic engineering
  - Genome editing with preassembled nucleases (e.g. functional PNP-complexes, no transgenes)
  - Tool for development of gene drives

RESULTS OF LITERATURE SURVEY /3

- Basic research aims
  - Screening approach for novel traits
  - Functional characterisation of genetic elements or combinations of alleles
  - Functional characterisation of homologous modifications in other plant species (model species - crops, wild relatives – crop species, related crop species)
  - New types of applications (Gene drives, development of epialleles, complex metabolic engineering)

- Applied development aims
  - Direct modification of elite lines of plants, e.g. vegetatively propagated plant species (GEd, cis/intra)
  - Faster development of traits in perennials with long generation times, e.g. fruit-trees (GEd, cis/int, accelerated breeding)
  - Specific transfer of traits from related plant species, e.g. wildforms (GEd, cis/intra)
  - Improvement of non-domesticated plant species (GEd, cis/intra, haploid induction)
  - Targeted, multiple modification (multiplexed GEd, molecular stacking by SDN-3)
  - Plant products without integrated transgenes (GEd, transgrafting, reverse breeding, RdDM)

RISK ASSESSMENT CONSIDERATIONS (SAM 2017)

- Effects due to **intended changes** present in the modified plant
  - Trait related effects:
    Herbicide resistance, disease resistance (viral, bacterial, fungal), compositional changes,
    enhanced fitness against environmental stressors, alteration of morphological or reproductive plant characteristics
- Effects due to **unintended changes** present in the modified plant
  - Method related effects:
    e.g. due to transformation (GM tools), nGM mechanism (off-target effects),
    other biotechnological methods (in vitro cultivation, regeneration)
- Effects due to **characteristics of the modified plant species** and its **interaction with the receiving environment**
  - (Major) crop species, other agricultural and ornamental plants, (fruit) trees
- Effects due to the **intended use of the modified plant**

INTENDED EFFECTS – TRAIT-RELATED CONSIDERATIONS

- Herbicide resistance (HR) against broadband herbicides
  - ALS-Inhibitors (OSR, potato, rice, maize, soy, tobacco), glyphosate (strawberries, flax, cassava, cotton), glufosinate, bialophos, 2,4-D (maize, tobacco)
- Disease resistance against plant pathogens
  - Bacterial and fungal pathogens (grapefruit, wheat, tomato, grapevine, apple, and rice)
  - Viral pathogens (cucumber)
- Compositional changes
  - sugar and starch content (potato and rice), lipid composition (*Camelina* and soybean), lignin (sugarcane), fragrance (rice)
- Enhanced fitness (environmental stressors), morphological or reproductive characteristics
  - Abiotic stress response (cold, drought, salinity)
  - Increased seed shatter resistance (oilseed rape), early maturation and facultative parthenocarpy (tomato), early flowering, larger fruit and more flower buds (tomato), *de novo* domestication (tomato)

Trait	Mechanisms	nGMs	Comp. dev.
Herbicide resistance (HR) (broadband herbicides)	Modification of target genes	GEd (SDN-1, SDN-2, ODM)	Class. mut., nat. selection
	Introduction of resistant alleles	GEd (SDN-3), cis/intra	GM
	Molecular stacking of multiple HR genes	GEd (SDN-3)	GM
Disease resistance (viral, bacterial, fungal pathogens)	Knockout of susceptibility genes (bact. and fungal path.)	GEd (SDN-1)	GM (RNAi)
	Knockout of viral host factors	GEd (SDN-1)	GM (RNAi)
	Expression of heterologous resistance genes and antimicrobials	Transgrafting, cis/intra	GM
Compositional changes	Knockout of biosynthetic enzymes	GEd (SDN-1)	GM (RNAi)
	Expression of biosynthetic genes	Cis/intra	GM
Altered env. fitness, morphology / reproduction	Knockout of endogenous genes	GEd (SDN-1) (multiplexed)	
	Expression of abiotic stress response genes	Transgrafting	GM

TRAIT-RELATED CONSIDERATIONS - ISSUES

- Limited knowledge and familiarity (no history of safe use)
  - Novel crops / agricultural plants (new species, wildforms)
  - Novel traits (phenotypes)
  - Changed agricultural management

- Unintended effects associated with intended changes
  - Pleiotropic effects
  - Unintended effects in non-transgenic plant parts (transgrafting)
  - Complex physiological effects of multiple changes (multiplexed GEd)
  - Complex regulatory effects on morphology, development and reproduction

Trait	Potential adverse effects	Comp. issues
Herbicide resistance (HR) (broadband herbicides)	Impacts on biodiversity due to herbicide use	GM
	Development of HR-weeds (selection, gene flow)	GM
	HR-volunteer plants in subsequent crops	GM
	Herbicide cocktail effects (residues, metabolites)	GM (stacks)
	Pleiotropic effects of overexpressed HR-genes	
Disease resistance (viral, bacterial, fungal pathogens)	Pleiotropic effects on plant development (e.g. multiple mlo-knockouts)	GM (RNAi)
	Evolution of pathogens (selection of resistant pathogens, secondary pathogens)	GM (IR)
	Effects on (soil) non-target organisms (antitimicrobials)	GM
Compositional changes	Animal and human health effects (nutritional effects, toxicity, allergenicity)	GM
	Environmental effects due to changed composition (herbivore attractivity, NTOs, decomposition)	GM
	Environmental effects of morphological changes (stability)	GM
Altered env. fitness, morphology / reproduction	Increased invasiveness of modified plant	GM
	Negative effects on protected relatives (gene flow)	GM
	Increased fitness of weedy relatives (gene flow)	GM
	Unintended effects of *de novo* domesticated plants (nutritional)	

CASE-SPECIFIC FRAMING OF RISK ASSESSMENT

Different categories of nGM applications

- nGM plants with traits and usage known from conventional approaches
  - Familiarity concerning effects upon use should be considered during assessment
- nGM plants with traits similar to existing GM plants and associated with comparable risk issues
  - e.g. herbicide resistance or disease resistance, compositional changes
  - previous experience with the risk assessment of comparable GMOs should be taken into account
- nGM plants with traits which have not yet been established and thus are novel
  - Similar approaches for risk assessment as implemented for GMOs should be applied

METHOD-RELATED CONSIDERATIONS

- Holistic assessment of unintended changes is required
  - Typically a combination of different methods is used (nGM(s), GM-methods, other breeding methods)
  - Unintended changes due to transformation procedures (GM methods, introduction of method-related components)
  - Unintended changes due to integration of unwanted genetic material (GM methods, transient introduction of genetic constructs)
  - Off-target effects genome editing – unintended as well as "intended" off-target effects (SDNs, ODM)
  - Unintended changes due to other required biotechnological methods (*in vitro* cultivation, protoplast technology, regeneration)

- Unintended changes at genomic locations other than the genomic target site(s)
  - Modifications usually not genetically linked to the desired trait(s)
- Unintended molecular changes in the vicinity of the intended site of modification
  - Different from the intended modifications, but tightly linked to the desired trait(s)

nGM CHARACTERISTICS RELEVANT FOR RA

- Combination of biotechnological and conventional methods
  - Different nGMs
  - nGMs, GM technology & other biotechnological methods
- Precision (specificity of GEd)
  - Potential to introduce off-target changes
  - Predictability of off-target changes
- Depth of Intervention
  - Single vs. multiple changes (multiplexed, serial changes)
  - Physiological effects of modified targets (specific vs. pleiotropic effects)
- Impact on time of development (observation)
  - Direct modification of elite lines, plants that are propagated vegetatively
  - Modification of plants with long generation cycles (trees)

CASE-SPECIFIC APPROACH TO RISK ASSESSMENT

- Case-specific risk assessment requirements taking into account
  - the nature of the developed trait,
  - unintended consequences of the modification introduced,
  - the available experience with comparable products, and
  - relevant protection goals specified by the respective countries.

- Appropriate molecular characterization to assess among other things
  - the unintentional presence of any transgenic inserts in the final product, and
  - the presence of off-target modifications and other unintended genetic changes, which might result in adverse phenotypic effects.

- Phenotypic characterization to specifically test parameters related to plausible risk issues associated with a particular nGM plant

CHARACTERISATION OF UNINTENDED EFFECTS

- Consider the specific characteristics of the nGM approach, and existing knowledge on potential for unintended changes/off-target activity
- Assess whether unlinked unintended modifications will be removed by crossbreeding steps
- Use robust bioinformatics tools to predict off-target changes and check predicted off-target activity against *in vitro* test data
- Use targeted sequencing to assess off-target changes linked to the desired modification(s), and whole genome sequencing in case of significant potential for unlinked off-target changes
- Assess whether any unintended changes might be functional relevant and significant in terms of biological effects
- Use targeted or untargeted phenotyping to assess the possible adverse effects resulting from unintended modifications/off-target

CHALLENGES FOR RISK ASSESSMENT

- Variety of methods and traits need to be considered

- No safety by default
  - The issue that certain changes could (hypothetically) be developed with conventional methods (or spontaneous mutation) is not indicative of product-safety
  - Small size genetic changes are not indicative of (environmental) safety
  - High precision of modification is not indicative of the safety of trait(s)

- Limited knowledge for assessment available
  - Novel traits and complex traits (Non-trivial physiological / phenotypical effects)
  - Limited predictability of (unintended) changes and effects

- Increased speed of development
  - Impacts observation and elimination of unintended changes

LIMPING ALONG AND LAGGING BEHIND: REGULATION

- Risks not correlated with specific types of techniques (e.g. SDN-1, SDN-2, ODM, SDN-3)
  - Rather with traits and characteristics of approach (complex, deep, fast, dirty)
  - Challenges for devising an appropriate regulatory approach (2-sets of criteria)

- Existing regulatory frameworks are not consistently addressing nGM-applications
  - Particularly they fail to consistently address the level of risk associated with different nGM-applications
  - Neither are proposed/implemented amendments increasing consistency

- Broad flexible framework probably best to address biosafety issues
  - Exclusion from biosafety regulation is not a good option – other applicable regulation is not well suited to address biosafety issues
  - General principles of GMO regulation frameworks are suitable
  - Case-specific „outcome-based" assessment strategies need to be developed

THANK YOU FOR YOUR ATTENTION!

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