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NEW GENETIC ENGINEERING: POSSIBLE UNINTENDED EFFECTS

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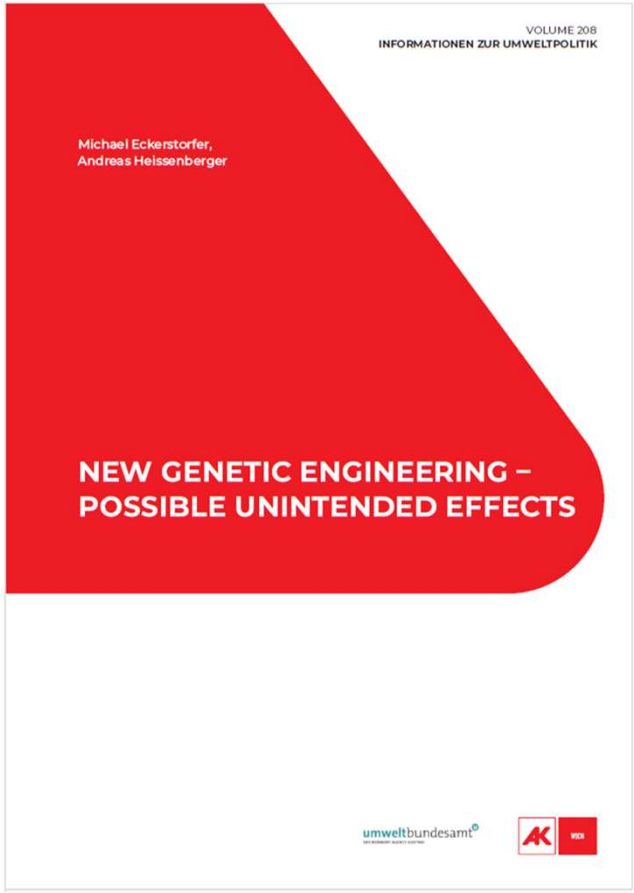
BACKGROUND

- Environment Agency Austria - Tasks regarding Biosafety
  - Environmental risk assessment and monitoring of GMOs (Directive 2001/18/EC & Reg. (EU) 1929/2003)
  - Studies addressing New Genomic Techniques (NGTs) e.g. Genome Editing since 2014:
    risk assessment, monitoring, detection/identification, considerations regarding sustainability, etc.
- New study addressing Unintended Effects of NGTs, on behalf of the Chamber of Labour, Vienna (Arbeiterkammer Wien)
  - https://emedien.arbeiterkammer.at/viewer/image/AC16982244/



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KEY ISSUES OF THE EUR. COMMISSION PROPOSAL

- The proposed regulation for NGT-plants is not harmonized with the existing legal requirements for GMOs
- No risk assessment would be required for > 90 % of all NGT-plants (NGT 1)
  - No assessment of unintended effects
- No monitoring would be required for NGT 1 plants
- No labelling and no traceability requirements for NGT 1 plants
- …

Substantial concerns about consumer protection (safety and freedom to chose), impacts on plant breeders and impacts on GM-free agricultural production

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LOWER SAFETY STANDARDS FOR NGT-PLANTS ?

- NGT – Category 1 plants (NGT 1)
  - Up to 20 different independent genetic changes: e.g. insertions/substitutions up to 20 base pairs, deletions of unlimited length, cisgenic changes – insertion of genes found in the further gene pool of the plant species (i.e. cisgenes)
  - Equivalence with conventional plants is assumed by the EC :
    current requirements for GM plants would no longer apply according to the EC proposal:
    - No mandatory risk assessment (and monitoring)
    - No assessment of unintended effects (as foreseen for GM-plants)
  - Combinations of NGT 1 plants by crossbreeding would again be considered to be NGT 1 plants
- NGT – Category 2 plants (NGT 2)
  - NGT plants with very complex, multiple changes (more/others than with NGT 1)
  - In principle, similar rules apply as for GMOs, however:
  - Less robust requirements will be introduced for risk assessment, monitoring, re-authorisation and detection methods than currently for GM-plants

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NO SCIENTIFIC JUSTIFICATION FOR THE EC PROPOSAL

- *NGT methods are considered to be more precise than classical methods of mutagenesis*
  - Precision is not absolute – unintended genetic changes are possible!
  - Precision is not synonymous with safety!
- *Equivalence criteria are based only on the number, type and size of genetic changes*
  - There is no scientific evidence that such criteria are indicators of safety! (Eckerstorfer et al. 2021, https://doi.org/10.3390/biotech10030010)
  - The different characteristics by NGT methods (localisation of mutations) is not taken into account! (Eckerstorfer et al. 2023, https://doi.org/10.3390/plants12091764)
- *The assumed equivalence with conventional plants is based on theoretical considerations only*
  - Most of the traits developed in NGT-plants are new! (Then 2022, vzbv-report_final_final.pdf)
  - For most NGT plants, there is no practical experience/data regarding their safety!
- *Unintended genetic changes are supposed to be similar to those in conventional plants*
  - Ignores the different technical sources of unintended genetic changes in NGT-plants

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WHEN WILL UNINTENDED EFFECTS OCCUR?

- Unforeseen “side effects” of intended genetic modifications
  - Many target genes have additional or complex functions, e.g. in metabolism, plant development, or in terms of their fitness in the environment.
- Unintended effects due to additional genetic changes
  - Imperfect removal of temporary genetic modifications for the expression of molecular tools for genome editing (e.g. CRISPR-Cas nuclease)
  - Mutations induced elsewhere in the genome than at the target sequence (off-target mutations)
  - Additional secondary changes adjacent to the target sequence (on-target mutations)
  - Mutations by *in vitro* methods used for the development of NGT-plants
    (plant cell culture, protoplast transformation, regeneration of plants from genetically modified cells)

Only when unintentional genetic changes result in adverse phenotypic effects relevant unintended effects will arise

- This is checked during case-by-case risk assessment!

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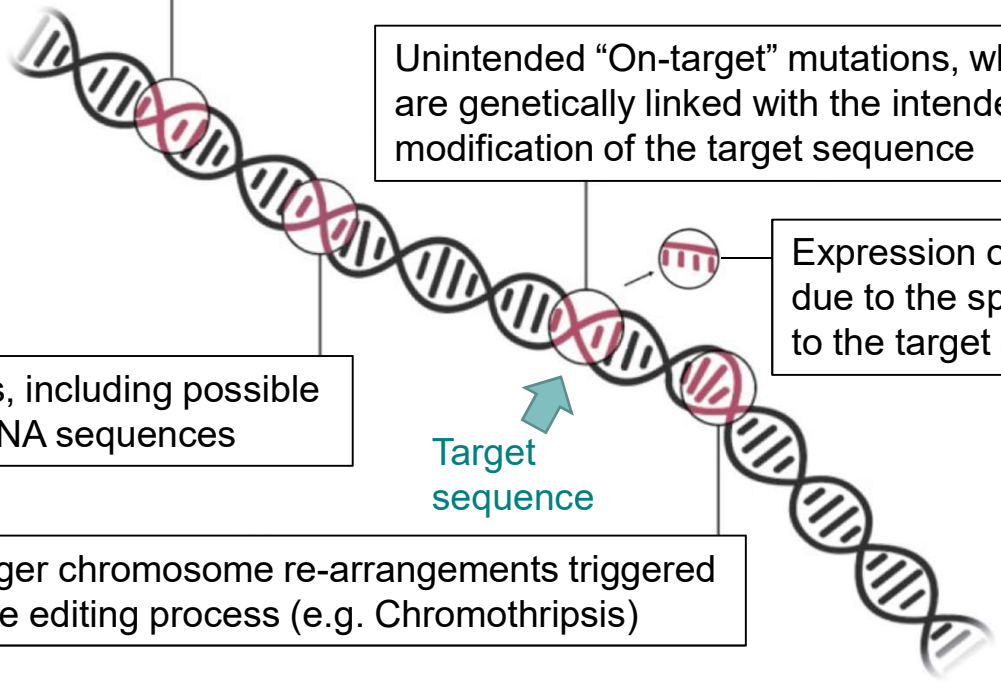
DIFFERENT TYPES OF UNINTENDED GENETIC CHANGES

Genetic changes due to insertion of transgenes necessary to express the molecular tools for genome editing (e.g. CRISPR-Cas)

Unintended “On-target” mutations, which are genetically linked with the intended modification of the target sequence

Expression of novel gene products due to the specific genetic changes to the target sequence

“Off-target” mutations, including possible insertion of foreign DNA sequences



Smaller or larger chromosome re-arrangements triggered by the genome editing process (e.g. Chromothripsis)

© modified from: Franziska Koller, FGU; What_do_we_really_know_about_NGT_plants.pdf (testbiotech.org)

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APPROACH USED FOR THE STUDY

1) Analysis of representative examples of NGT-plants with relevant characteristics based on:
   - Information about these NGT-plants from the scientific literature
   - Information from non-EU authorities on these NGT-plants
   - Analysis by European Authorities (EFSA)

2) Evaluation of the existing level of knowledge concerning unintended effects based on:
   - Systematic reviews of published information concerning Unintended Genetic Changes in NGT-plants
   - Information on Unintended Genetic Changes by NGT methods found in overall scientific literature

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NGT-PLANTS ADDRESSED AS CASE STUDIES

- NGT-tomatoes with an increased content of gamma-aminobutyric acid (GABA)
  - NGT 1, “functional food” with altered composition
  - Described in scientific literature; available in Japan since 2023
- NGT-wheat with a reduced gluten content
  - NGT 2 (> 30 modified genes), plant with complex alteration of composition
  - Described in scientific literature; analysed by EFSA (Naegeli et al., 2021)
- NGT-rice with increased tolerance against climate and salt stress
  - NGT 1, increased resilience (fitness) against environmental stress (higher salinity in the soil)
  - Described in scientific literature
- “*De Novo domesticated*” NGT-tomatoes with increased disease resistance
  - NGT1, Change of composition, shape, development/reproduction properties
  - Described in scientific literature; analysed by EFSA (Mullins et al., 2022)

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UNINTENDED EFFECTS CONSIDERED FOR CASE STUDIES

- NGT-tomatoes with an increased content of gamma-aminobutyric acid (GABA)
  - Potentially adverse medical effects on vulnerable populations
  - Possible effects on microorganisms and insects; unintended effects on plant shape and growth
- NGT-wheat with a reduced gluten content
  - Possible intolerance of people with celiac disease; negative effects of the introduced genetic changes
  - Potentially reduced resilience to environmental stress
- NGT-rice with increased tolerance against climate and salt stress
  - Indirect changes in composition and food safety
  - Possible loss of yield in the absence of environmental stress
- "*De Novo domesticated*" NGT-tomatoes with increased disease resistance
  - Possible differences regarding the wholesomeness to currently consumed tomatoes
  - Possible negative effects due to the untried and untested genetic background of the parental wild plant

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LEVEL OF KNOWLEDGE REGARDING “UE” IN NGT-PLANTS

- Current knowledge is generally limited
  - UE are rarely studied systematically
  - Accumulating evidence concerning occurrence of UE in scientific literature (Then 2022, vzbv-report_final_final.pdf)
- Research focuses on “off-target” changes introduced by NGT methods
  - Other types of UE less frequently investigated (Chu & Agapito 2022, https://doi.org/10.3390/plants11212997)
  - Focus on “off-target” changes at individually selected locations (with sequence similarities)
- Investigations are carried out for purposes other than risk assessment
  - For method optimisation instead of risk assessment and at a time unsuitable for RA (immediately after genome editing) (Sturme et al. 2022, https://doi.org/10.1021/acsagscitech.1c00270)
- Focus on Genetic Changes and Not Unintended Effects
  - No assessment of phenotypic effects of identified genetic changes (Sturme et al. 2022, https://doi.org/10.1021/acsagscitech.1c00270), no reliable conclusions concerning the occurrence of adverse effects can be drawn

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RELEVANT CONSIDERATIONS FOR “UE” IN NGT PLANTS

Human Gene Therapy

- Very high precision is required
- Extensive research into unintended genetic changes
- Other systems than CRISPR-Cas9 are developed to minimize UE:
  - CRISPR-associated transposases avoid double-strand breaks in DNA: Lampe *et al.* (2024). Nat. Biotechnol. 42, 87–98

Animal Biotechnology

- NGT Hornless Cow (Recombinetics, Inc.)
- Risk assessment incl. Molecular characterisation by US-FDA:
  - Unintended integration of plasmid sequences and foreign DNA was identified: Norris et al. (2020). Nat. Biotechnol. 38, 163–164

Plant Biotechnology

- Efficacy of modification vs. minimising off-target activity
- Multiplexing is achieved with use of less-precise NGT methods
- Less subsequent crossbreeding for certain plants (e.g. with longer reproductive cycles as NGT-trees)
- Modification of elite plant varieties to speed up breeding
  - Off-target edits may be retained
  - Mol. characterisation and phenotypic risk assessment is required!

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RECOMMENDATIONS

- An appropriate, comprehensive risk assessment is necessary for NGT-plants !
  - Potential risks are not limited to certain groups of NGT-plants (e.g. NGT 2), but will occur in a case-specific manner!
- Similar as for GMOs, the risk assessment of NGT-plants must be carried out case-by-case!
  - Addressing plausible risk aspects related to the new traits and all biotechnological methods used for development
- Risk assessment must take into account the possible unintended effects
  - Relevant aspects are the breeding history of the respective NGT-plants, the degree of method optimization and the level of knowledge about the modified genes and their functions in the NGT-plant
- Other undesirable effects of the proposed regulation must be avoided as well:
  - The freedom of choice must be maintained for consumers – this needs labelling of food and feed products!
  - No additional burdens should be placed on production systems that won´t use GMOs or NGT-plants!
  - Access to plant material for breeding must not be restricted!

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