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Education and Research EAER

Agroscope

Studying unintended effects confuses the nontarget risk assessment

Jörg Romeis and Michael Meissle

Environmental risk assessment

- Formulation of plausible risk hypotheses (problem formulation)
- Test those risk hypotheses
- If necessary, conduct studies that are reproducible and provide reliable data to address the risk hypotheses
- Collect only data where risk assessors agree how to interpret the data and how to use them in the risk assessment
- New data should decrease the level of uncertainty

Comparative approach

- Compare the GM crop with its non-GM conventional counterpart
- Establish what is different in the GM plant under assessment
- Based mainly on data from three different sources:
  - molecular characterization
  - compositional analysis
  - agronomic comparison
- Establish the biological relevance of these differences

Garcia-Alonso 2010 IOBC/WPRS Bulletin 52, 57-63

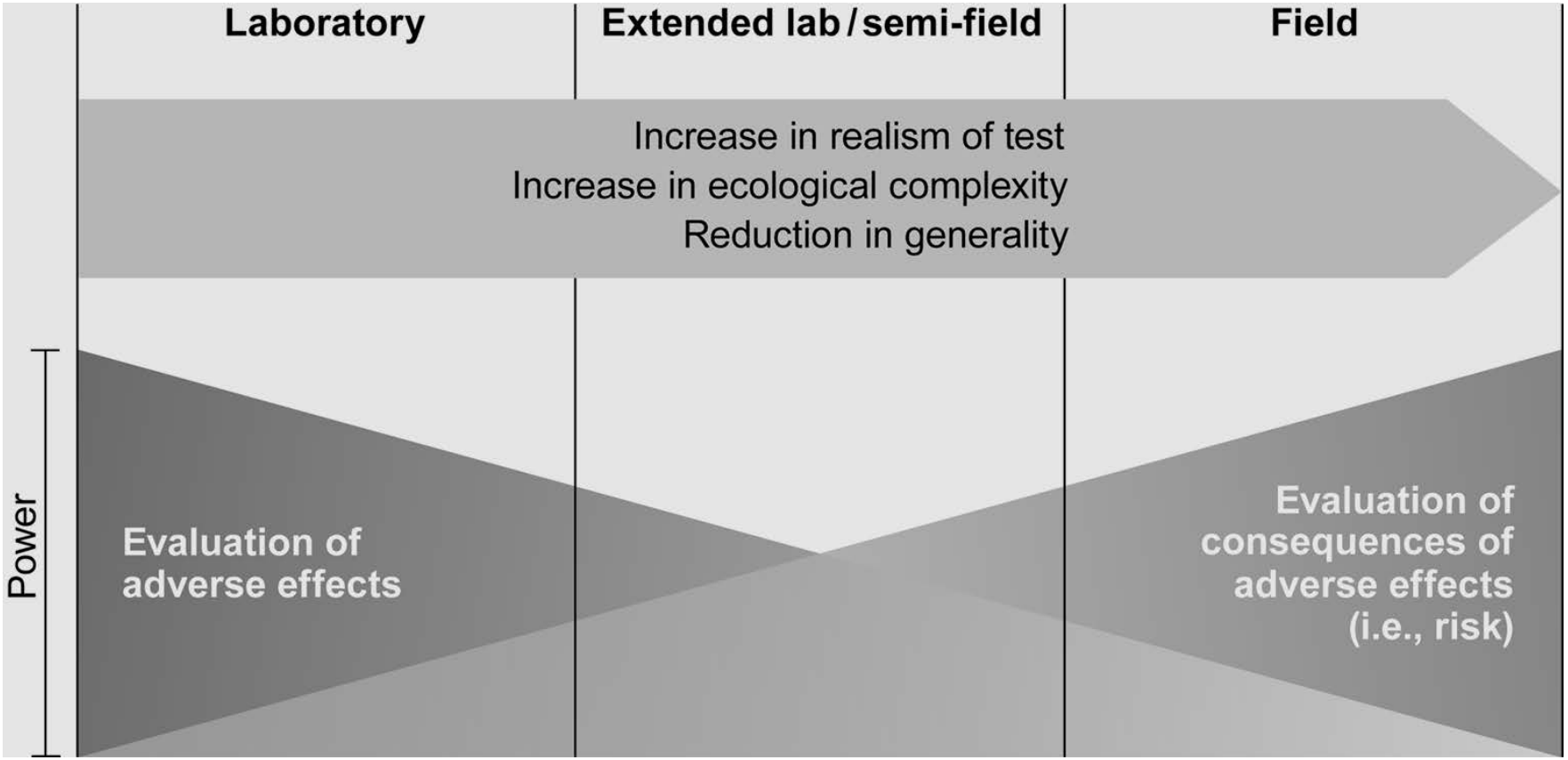
Non-target risk assessment

Two sources of risk

- The intended change (e.g., production of a Bt toxin) may cause (anticipated) unintended effects on NTOs
- Transformation of the plant may introduce unintended changes that may lead to (unanticipated) unintended effects on NTOs
  - addressed by a „weight of evidence“ approach *(molecular, composition, agronomic)*
  - identified relevant differences need to be assessed

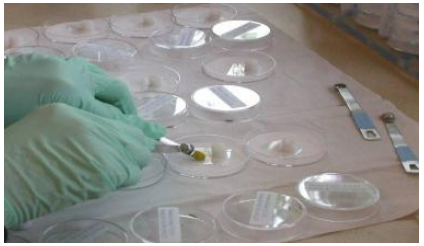
Assessment of anticipated unintended effects

Risk hypothesis: *„The expressed protein is not toxic to non-target arthropods at the concentration present in the field.“*



Romeis et al. 2013 Chemosphere 90, 901-909

Laboratory non-target studies



Powerful test of the risk hypotheses

- Select test species according to their relevance, potential sensitivity, availability/ reliability
- Good characterization of the test substance
- Worst-case exposure conditions using purified protein as test substance (safety margin)
- Good interpretability of data: test stressor in isolation, negative/ positive control treatments
- High replication, high statistical power

High confidence in a conclusion of no adverse effects!

Romeis et al. 2011
Transgenic Research 20, 1-22
Romeis et al. 2013
Chemosphere 90, 901-909

Assessing unanticipated unintended effects

EFSA (2010) is considering data from:

- Molecular characterization, compositional analysis, agronomic and phenotypic characterisation
- **GM plant-NTO interactions**
  - **Field studies**
  - **Laboratory Tier1b studies (using GM plant material)**

Reasoning: Compositional analyses do not necessarily target specific metabolites known to be involved in NTO-plant relationships

Devos et al. 2012 IOBC/WPRS Bulletin 73, 37-43
Devos et al. 2014 Transgenic Research 23, 1-25

Assessing unanticipated unintended effects

Possible risk hypothesis:

„The transformation has not caused changes in specific metabolites known to be involved in NTO-plant relationships.“

Difficulties:

- we usually do not know which metabolites are involved in these interactions
- Different metabolites are likely to affect different NTOs differently

Challenges with Tier1b studies

- Choice/ characterization of test substance
  - choice of comparator
  - production of plant material
  - selection of plant tissue
  - Bi- or tri-trophic tests with natural enemies
- Selection of test species
- Data interpretation

Choice of comparator

EFSA (2010): use „*near isogenic non-GM materials*“

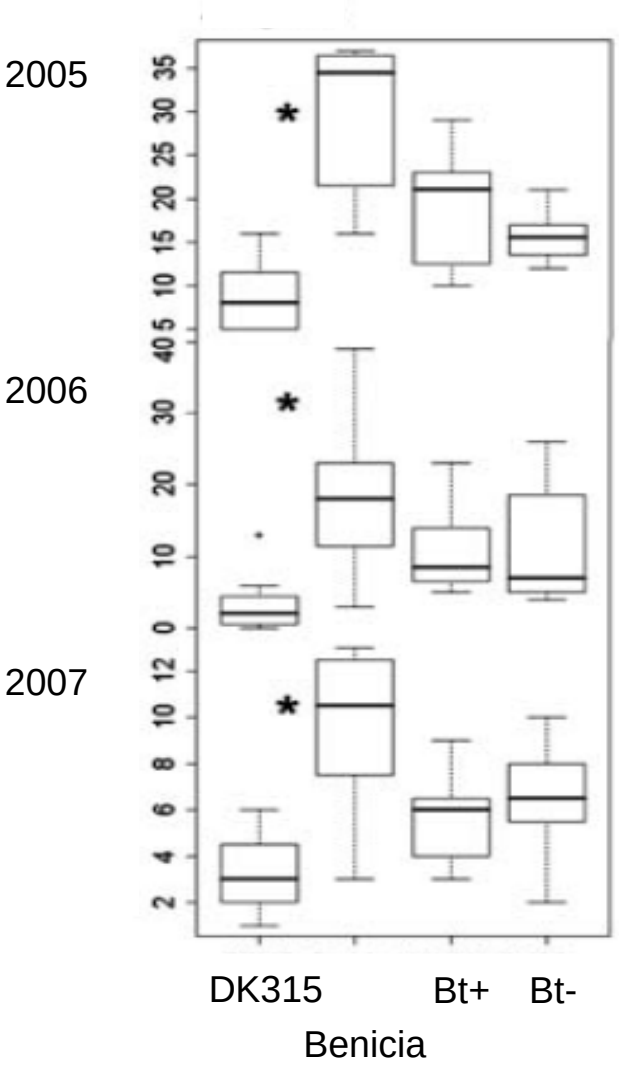
- A „near isogenic“ differs in more than the transgene from the GM line
- A „near isogenic“ line may not be available

Example: stacks that are produced by conventional crossings

Consequence: Any effect observed could be due to factors other than the presence of the transgene

Variation among plant varieties

Number of *Trigonotylos caelestialium* nymphs per plot



http://www.koleopterologie.de

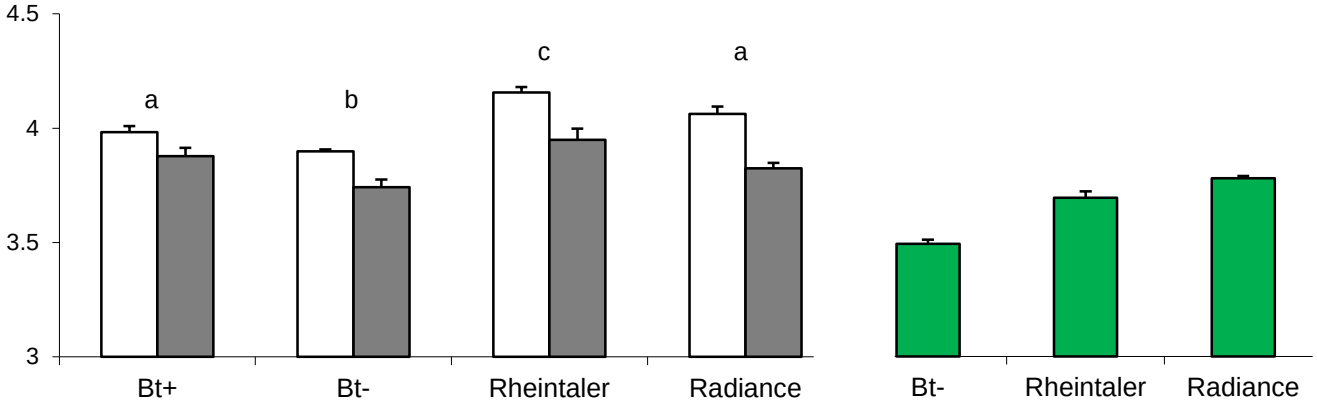
- Which GM variety to test?
- What is the baseline?
- Which effect is relevant?

Rauschen et al. 2009 Transgenic Research 18, 203-214

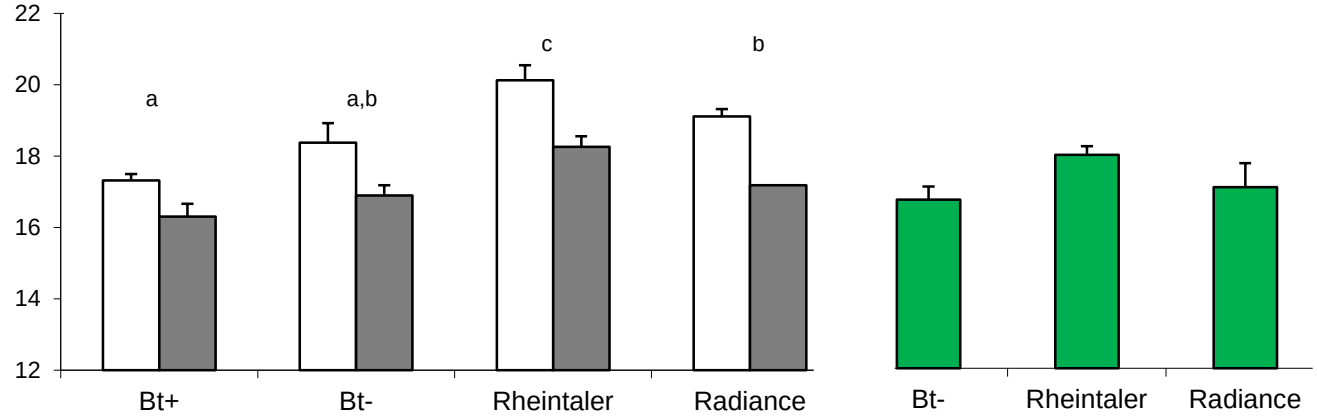
Production of test material

Maize pollen analyses *Romeis et al., unpublished results*

Nitrogen (% + SE)



Total protein (% + SE)



Field
Semi-field
Glasshouse

Significant differences

- between environments (semi-field/ glasshouse)
- among maize varieties

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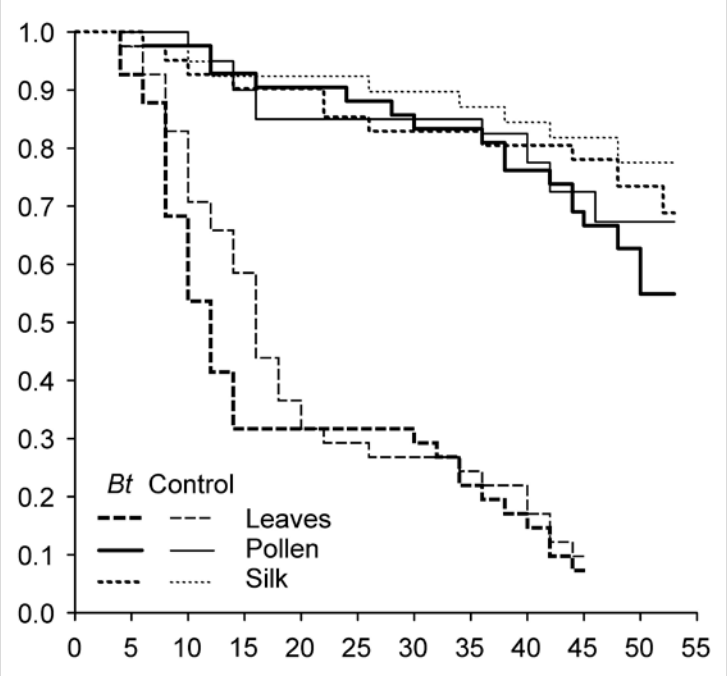
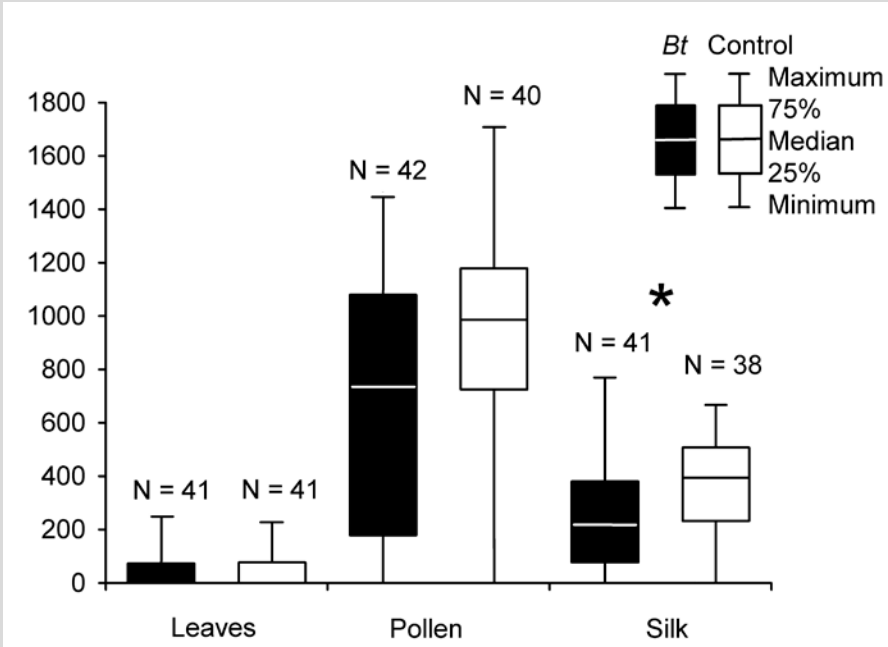
Selection of plant tissue

EFSA (2010): Tier1b studies should „… *reflect realistic exposure conditions* …“

Female survival (proportion)



No. of eggs per female



The outcome of the test might differ with the plant material that was used

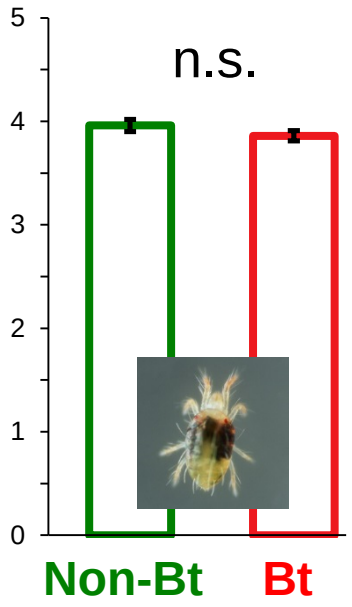
Meissle et al. 2011 Pest Management Science 67, 807-814

Selection of test material

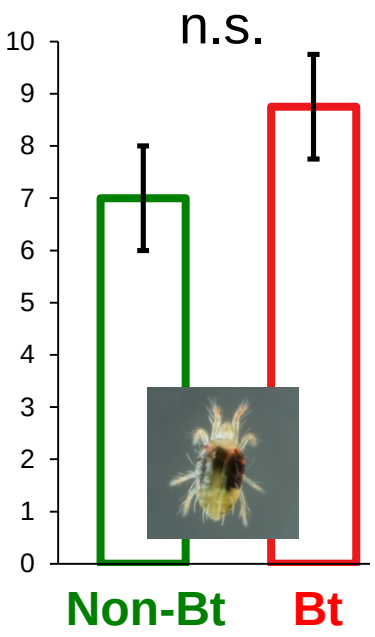
Obrist et al. 2006 Experimental and Applied Acarology 38, 125-139

Predatory mite *Neoseiulus cucumeris*

Female development time (d ± SE)



Number of eggs per female (± SE)



5.1 µg Cry1Ab / g FW

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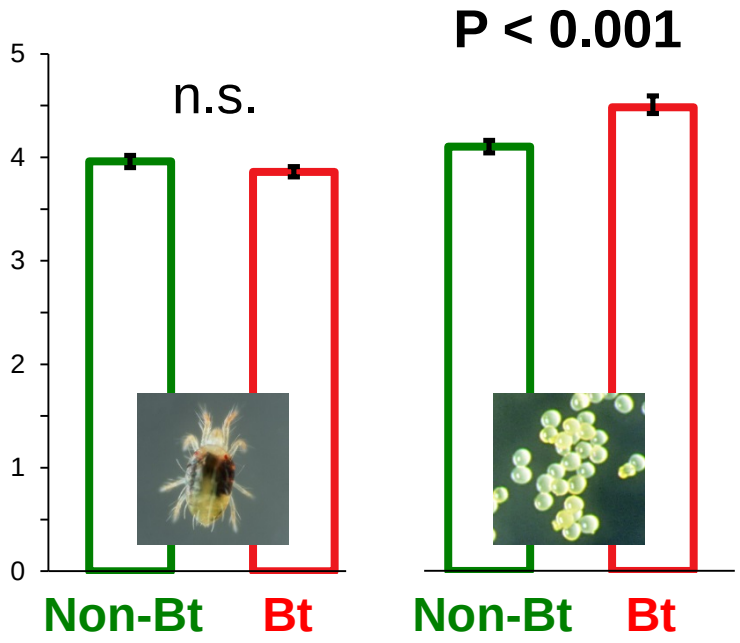

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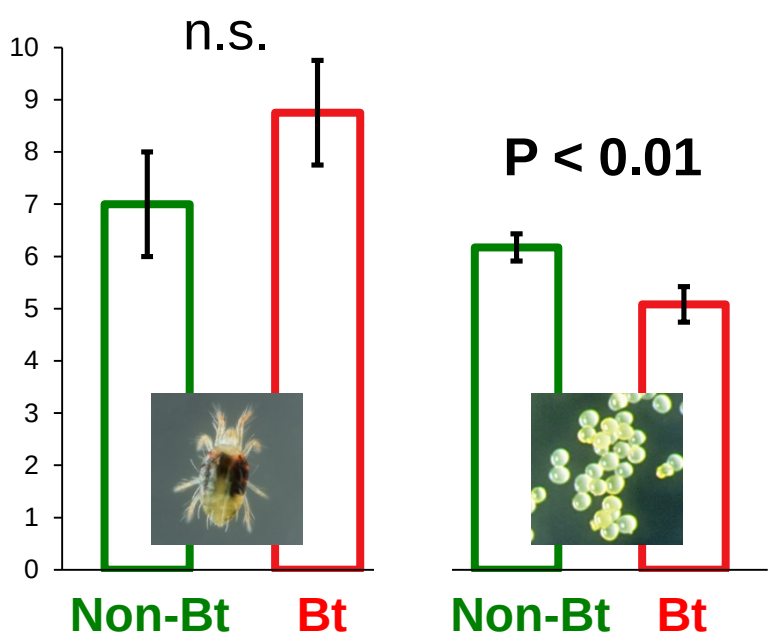

5.1 µg Cry1Ab / g FW

Cry1Ab in ng range

Bi- and tri-trophic test gives different results

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Selection of test species

Selection criteria *(Romeis et al. 2013)*:

1 Relevance	representative of valued taxa/ functional groups most likely to be exposed to the stressor
2 Potential sensitivity	species most likely to be sensitive to the test compound
3 Availability/ reliability	availability of test insects and validated test protocols

Risk hypothesis I: *„The expressed protein is not toxic to non-target arthropods at the concentration present in the field.“* **1 & 2 & 3**

Risk hypothesis II: *„The transformation has not caused changes in specific metabolites known to be involved in NTO-plant relationships.“* **1(in parts) & 3**

Conclusions

Avoid testing for unanticipated unintended effects

- … if stressor of concern is not known
- Impossible to separate plant background effects from effects caused by the stressor
- Data are difficult/ impossible to interpret
- Lack of baseline data
- Impossible to account for plant-to-plant (variety) effects. Introduce a variety-specific regulation?
- Data are only relevant to a specific GM variety-NTO system (lack of data transportability)

Conclusions

- Data are likely to confuse rather than to add certainty to the risk assessment

Suggestion

Focus on molecular characterization, compositional analysis, agronomic/ phenotypic characterization to identify stressors of concern (*including unintended effects*) and to develop plausible risk hypotheses that can then be tested

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