FH CAMPUS WIEN
UNIVERSITY OF APPLIED SCIENCES

DIE HOCHSCHULE, DIE MEHR KANN.

Which in-vitro bioassay is the most suitable?

SECTION PACKAGING AND RESOURCE MANAGEMENT

Overview

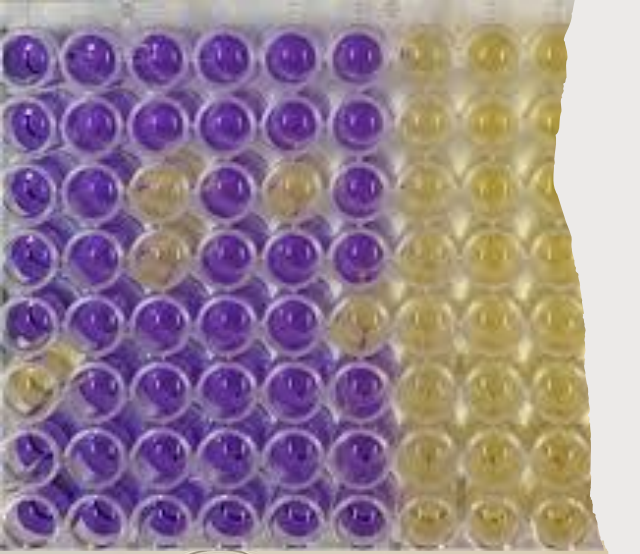
>Overview – types of *in vitro* bioassays for genotoxicity

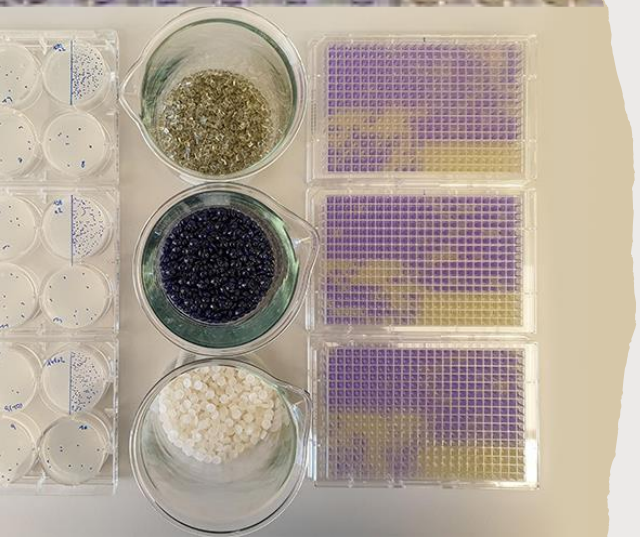
>Factors that influence the bioassay selection for NIAS safety assessment

>Limits of biodetection

>Comparison of different assay types

>Recommendations and overall results





In vitro bioassays for genotoxicity testing

- Tests based on mammalian cells
  - Micronucleus assay
  - Chromosomal abberation test
  - Multiple reportergene assays
- Tests based on bacteria
  - Ames test
  - Rec Assay
  - umuC
- Tests with fungi and yeasts

Factors that influence bioassay selection (for NIAS)

> Availability and cost

> Existing database and regulatory acceptance

> Covering of relevant endpoints for NIAS

> Limits of biodection

> Tolerance of toxic sample effects

> Sensitivity and specificity

> Ease of use and reproducability

Availability and cost

Costs for performing assays under GMP:

>Bacterial reverse mutation assay $5,800

>Mammalian erthrocyte micronucleus test $25,800

>In vitro mammalian chromosomal aberration test $31,600

>Reportergene assays vary widely and information is hard to find

>Test kits might be available starting at ~$ 1000, a lot of labor and specialized setups are required

Existing data and regulatory acceptance

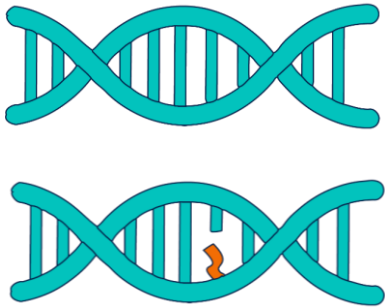
>Novel assays have little to no published data

>Several assays are highly recognized and can be found in multiple international guidelines (e.g. ICH M7, OECD TG487…)

>Well recognized assays for testing of genotoxicity are:
  >Ames test (primary test in most guidelines)
  >Micronucleus assay (mammalian cells)
  >Chromosomal abberation assay (mammalian cells)

Relevant endpoints for NIAS

1. **Direct DNA-reactive / mutagen:** change DNA sequence

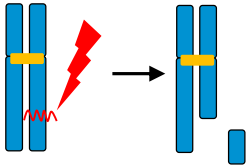


Mutation

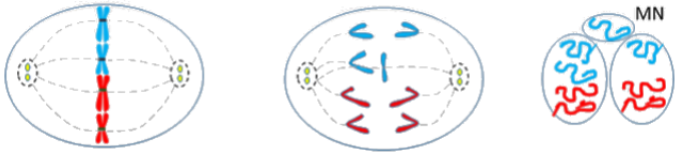
- Very low safety threshold (TTC)
- Detection: Ames-Test (bacteria based)

2. Clastogenic/Aneugenic: indirect DNA changes on chromosomal level

- **Clastogene:** Chromosome breaks (Deletionen, Insertionen, chromosomal rearrangements)



- **Aneugenic:** damages during the cell division/ mitotic spindle



Limits of biodetection

>One of the main research focuses of the Migratox project

>Overall, Ames MPF better than Ames better than Mammalian cell-based assays

PeerJ

HepGentox: a novel promising HepG2 reportergene-assay for the detection of genotoxic substances in complex mixtures

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Suitability of the Ames test to characterise genotoxicity of food contact material migrates

Bernhard Rainer[a], Elisabeth Pinter[a], Thomas Czerny[a], Elisabeth Riegel[a], Christian Kirchnawy[b], Maricel Marin-Kuan[c], Benoît Schilter[c] and Manfred Tacker[a]

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Review

Evaluation of the Suitability of Mammalian *In Vitro* Assays to Assess the Genotoxic Potential of Food Contact Materials

Elisabeth Pinter [1,*], Bernhard Rainer [1], Thomas Czerny [1], Elisabeth Riegel [1], Benoît Schilter [2], Maricel Marin-Kuan [2] and Manfred Tacker [1]

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toxics

MDPI

Article

Direct Comparison of the Lowest Effect Concentrations of Mutagenic Reference Substances in Two Ames Test Formats

Bernhard Rainer [1,*], Elisabeth Pinter [1], Lukas Prielinger [1], Chiara Coppola [1], Maricel Marin-Kuan [2], Benoit Schilter [2], Silvia Apprich [1] and Manfred Tacker [1]

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Sensitivity and Specificity

> Endpoint: rodent carcinogenicity
  > Different to DNA reactive genotoxicity!
  > Ames scores worst because of clastogen/aneugen effects

> Sensitivity: the ability to detect a carcinogenic substance as positive

> Specificity: the ability to detect non-carcinogens as negative

> Data is for pure substances only!

> Substance selection and quantity has a major impact on results

Published performance parameters for a selection of *in vitro* genotoxicity assays

Test name	Sensitivity (%)	Specificity (%)	References
1.Regulatory			
Bacterial reversion (Ames)	60	77	Kirkland *et al.*, 2005
Chromosome aberrations	70	55	Kirkland *et al.*, 2005
Mammalian mutation	81	48	Kirkland *et al.*, 2005
2.Screening			
Bacterial			
SOS Umu C	62	72	Reifferscheid and Heil, 1996
Ames MPF	58	63[a]	Kamber *et al.*, 2009
Yeast			
RAD54-GFP	39	82	Knight *et al.*, 2007
DEL	86	80[a]	Brennan and Schiestl, 2004
Mammalian			
MNT	81	54	Kirkland *et al.*, 2005
GADD45a-GFP[b]	87	95	Hastwell *et al.*, 2009

Other points:

>Tolerance of toxic sample effects:
  >Bacterial tests have a comparatively higher tolerance for organic solvents, antibacterial compounds are an issue
  >Toxicity must be quantifyable

>Ease of use and reproducibility:
  >In vitro assays for genotoxicity are never easy to use
  >Multiple assays have shown to produce reliable results across multiple laboratories
  >Ames test has the most data to back it up

Several in vitro bioassays for NIAS safety assessment*

Criteria	Micronucleus Assay	Chromosome Abbarations	Mammalian Rep.gen.	Ames Test OECD	Ames MPF	umuC
Cost	--	--	+	+	++	+++
Acceptance	++	++	-	+++	+	-
Endpoints	-	-	-	+	+	-
LOBD	-	-	+	+	++	++
Toxic samples	-	-	-	+	++	+
Sens./Spec.	+	+	+	+	+	+
Ease/Rep**	--	--	+	-	+	+

*Results are based on the applicability for NIAS in the context of the migratox project, tests that score low are not „bad" tests overall
**Highly depends on experience, laboratory infrastructure and potential automatization

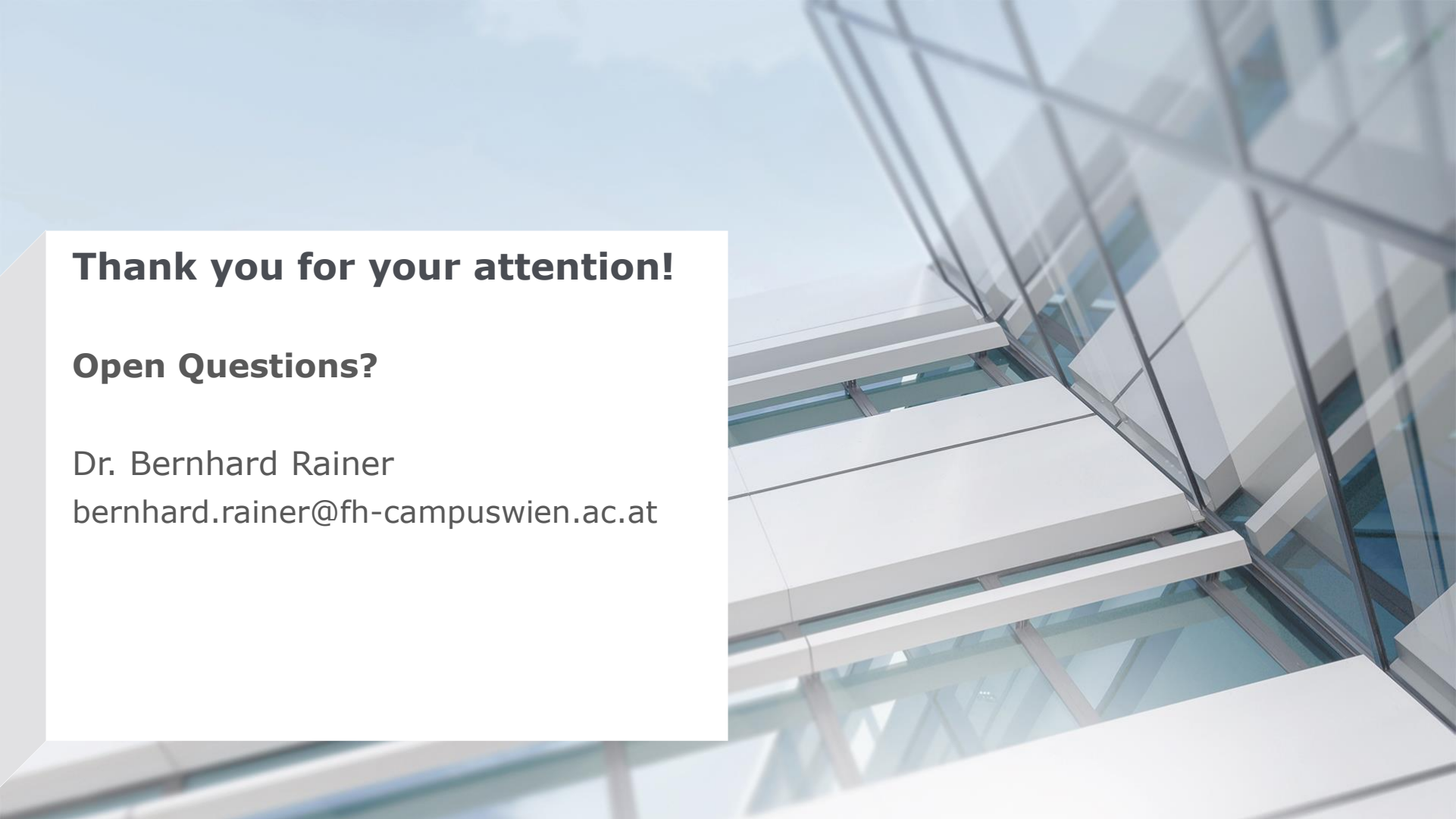
Discussion

>A **wide variety of assays** is available – presented list is not exhaustive!

>The assay must be chosen based on a **variety of factors** specific to the issue that is addressed

>In **our use case**, the Ames MPF assay wins out

>**SenseAmes** might be the natural „successor" and offer even better LOBDs and additional advantages!



Thank you for your attention!

Open Questions?

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