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DIE HOCHSCHULE, DIE MEHR KANN.

The Sense Ames: Optimization of a miniaturized Ames test

Thomas Czerny, Lukas Prielinger: Migratox Industry board meeting, 24.01.2024

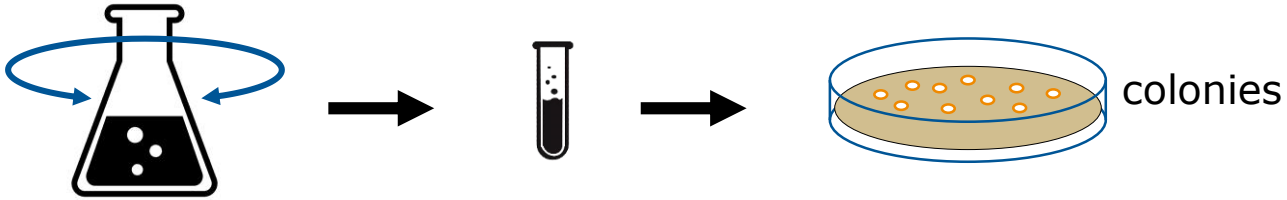
Ames Test

Salmonella bacteria with mutation in his pathway, strains TA100 and TA98

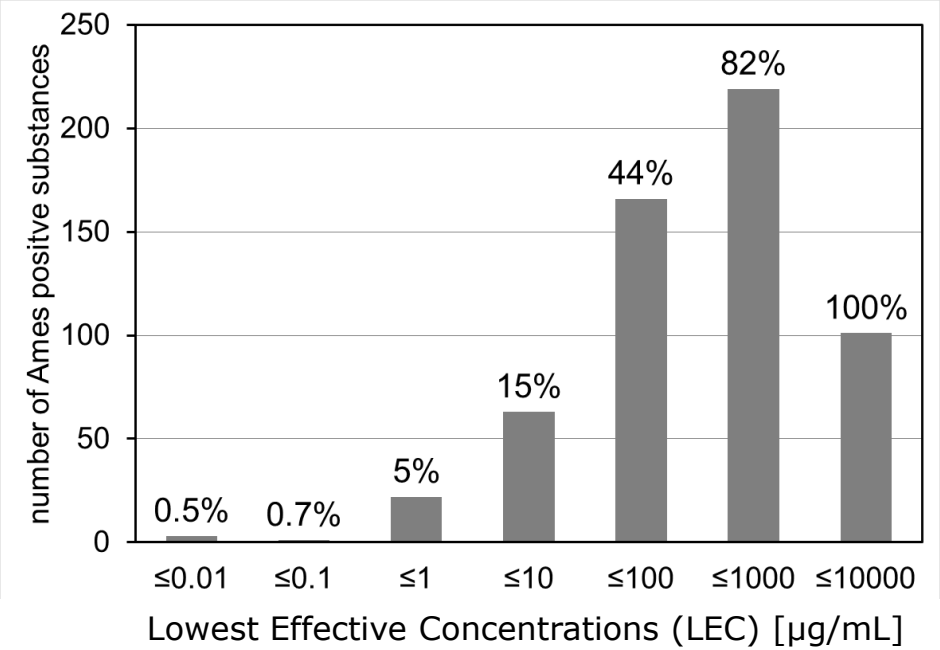
O/N culture with external his

exposure -> reverse mutations

medium without his: only mutated bacteria will grow



Detection limits of the Ames Test



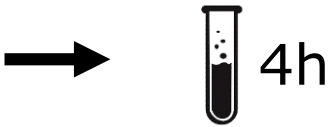
- Bioassays detect genotoxins at vastly different concentrations
- That critically affects their application for mixtures
- We analysed the detection limits in a large collection of Ames experiments from the NTP
- Majority of substances detected only at high concentrations with plate assay
- **Urgent need for optimization of the Ames test for mixtures**

Sense Ames

high reproducibility

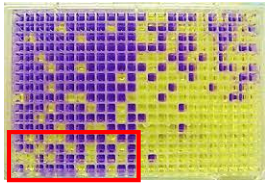
frozen bacteria
-80°C

high sensitivity



adaptations of
buffers, media, ...

high performance



2 µL volume
high throughput

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Comparison of assays

	Plate Assay	Sense Ames
	colonies on agar plate	liquid culture in 384 well plates
volume exposure	650 µL	20 µL
sample volume	50 µL	2 µL
exposure time	20-60 min	4 hours
bacteria	fresh o/n culture	frozen
reproducibility	low	high

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Performance of the experiments

	Plate Assay	Sense Ames
preparation day before	pour agar plates inoculate culture	-
experiment: samples/day/person	48 samples	288 samples
evaluation after 2 days	colony counting	fully automatic plate reader

Improvement of detection limits

Fold improvement of detection limit with Sense Ames compared to plate assay

		Concentration	Amount
2-Acetylaminofluorene	2AAF	12,1	1636
Aflatoxin B1	AFB1	7,8	1053
Benzo-α-pyrene	BaP	35,9	4844
Cisplatin	CisP	22,2	3002
2,4-Diaminotoluene	DAT	13,5	1823
7,12 Dimethylbenzanthracene	DMBA	733	98988
N-Ethyl nitrosourea	ENU	4,2	567
2-Amino-3-methyl-3H-imidazo[4,5-f]quinoline	IQ	39,8	5375
Methyl Methanosulphonate	MMS	1,8	242
4-Nitroquinoline-N-Oxide	4NQO	54,0	7289
2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine	PhIP	62,9	8486
Dimethylcarbamoyl chloride	DMC	5,5	737
Glycidol	Gly	14,7	1988
Hydrazine	Hyd	328	44294
Dimethyl Sulfate	DS	0,2	20
Acridin orange	AO	31,1	4194
2-Aminoanthracen	2AA	95,7	12919
2-Aminofluorene	2AF	3,0	402
Triglycidyl Isocyanurat	TGI	11,5	1559
2-Nitropropane	NP	3,3	440
Ethidium Bromide	EtBr	195	26320
Quercetine	Q	2,0	273
Phenylglycidylether	PGE	2,5	335
2-Nitrofluorene	2NF	23,6	3186
average		**71**	**9582**

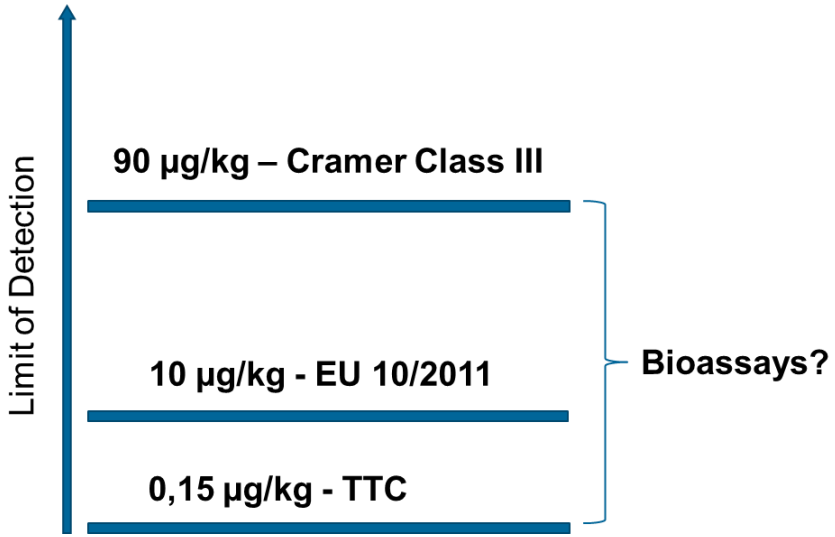
Results Sense Ames compared to plate assay

experiments with 2 strains: TA98 and TA100 +/- S9

71 x improvement for concentration

9600 x improvement for amount of substance

Strategy - Analysis of NIAS in FCMs



>Lowest effect concentration (LEC) of <0.15 µg/kg is required

>Unknown substances are classified as potentially DNA-reactive *

>Bioassays together with chemical analysis still need to be improved to achieve the required detection limits

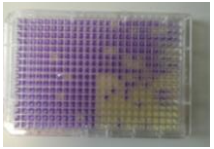
***Source**: EFSA (2019):Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment, https://doi.org/10.2903/j.efsa.2019.5708

Method - Ames MPF vs. Sense Ames

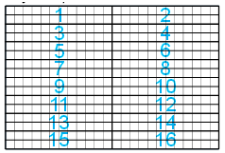
>Direct comparison of two Ames test formats (Ames MPF vs. Sense Ames)
>24 recycled plastic sample extracts (SA01-SA24) were tested
>Two conditions: TA98 with and without metabolic activation (S9)
>At least two runs for each sample (duplicate determination)
>Confirmation of Ames MPF results and comparison of LEC values

Migration: 95% Ethanol
10 days, 60 °C

Concentration (300x)
and solvent exchange
to DMSO



Ames MPF



Sense Ames

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Results – Ames MPF vs. Sense Ames

Sample Code	Ames MPF TA98 -S9		Sense Ames TA98 -S9		Ames MPF TA98 +S9		Sense Ames TA98 +S9	
	Result	Based on dilution	Result	Based on dilution	Result	Based on dilution	Result	Based on dilution
SA01	-	-	-	-	-	-	-	-
SA02	-	-	-	-	-	-	-	-
SA03	-	-	-	-	-	-	+	1:2
SA04	-	-	-	-	+	1:1	+	1:2
SA05	-	-	-	-	+	1:1	+	1:2
SA06	-	-	-	-	-	-	+	1:1
SA07	-	-	-	-	-	-	-	-
SA08	-	-	-	-	-	-	-	-
SA09	-	-	-	-	-	-	-	-
SA10	-	-	-	-	-	-	+	1:1
SA11	-	-	+	1:1	+	1:256	+	1:256
SA12	+	1:2	+	1:8	+	1:256	+	1:256
SA13	-	-	+	1:2	+	1:16	+	1:8
SA14	-	-	+	1:1	+	1:4	+	1:16
SA15	-	-	-	-	+	1:4	+	1:8
SA16	-	-	-	-	-	-	-	-
SA17	-	-	-	-	-	-	-	-
SA18	-	-	-	-	+	1:8	+	1:16
SA19	-	-	+	1:8	+	1:32	+	1:16
SA20	-	-	-	-	+	1:8	+	1:8
SA21	-	-	-	-	-	-	-	-
SA22	-	-	-	-	+	1:16	+	1:32
SA23	-	-	-	-	+	1:8	+	1:16
SA24	-	-	-	-	+	1:1	+	1:2

Experimental work and data by Thomas Barila, BSc

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Comparison - Ames MPF vs. Sense Ames

	Ames MPF	Sense Ames
Exposure volume	250 µL	20 µL
Sample volume	10 µL	2 µL
Exposure time	90 min	4 hours
LEC improvements compared to the OECD plate Ames	~6-fold average improvement of detection limits *	~70-fold average improvement of detection limits
Positivity rate of recycled polymer extracts	51/119 sample extracts were tested positive in the strain TA98 +S9 **	~8% more sample extracts were tested positive

***Source**: Rainer et al. 2021: Direct Comparison of the Lowest Effect Concentrations of Mutagenic Reference Substances in Two Ames Test Formats, https://doi.org/10.3390/toxics9070152

****Source:** Mayrhofer, Prielinger et al. 2023: Safety Assessment of Recycled Plastics from Post-Consumer Waste with a Combination of a Miniaturized Ames Test and Chromatographic Analysis, https://doi.org/10.3390/recycling8060087

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Conclusions – Sense Ames

>SenseAmes is novel Ames micromethod with superior detection limits

>High throughput method with fully automatic evaluation on plate reader

>Freezing of bacteria strongly improves reproducibility

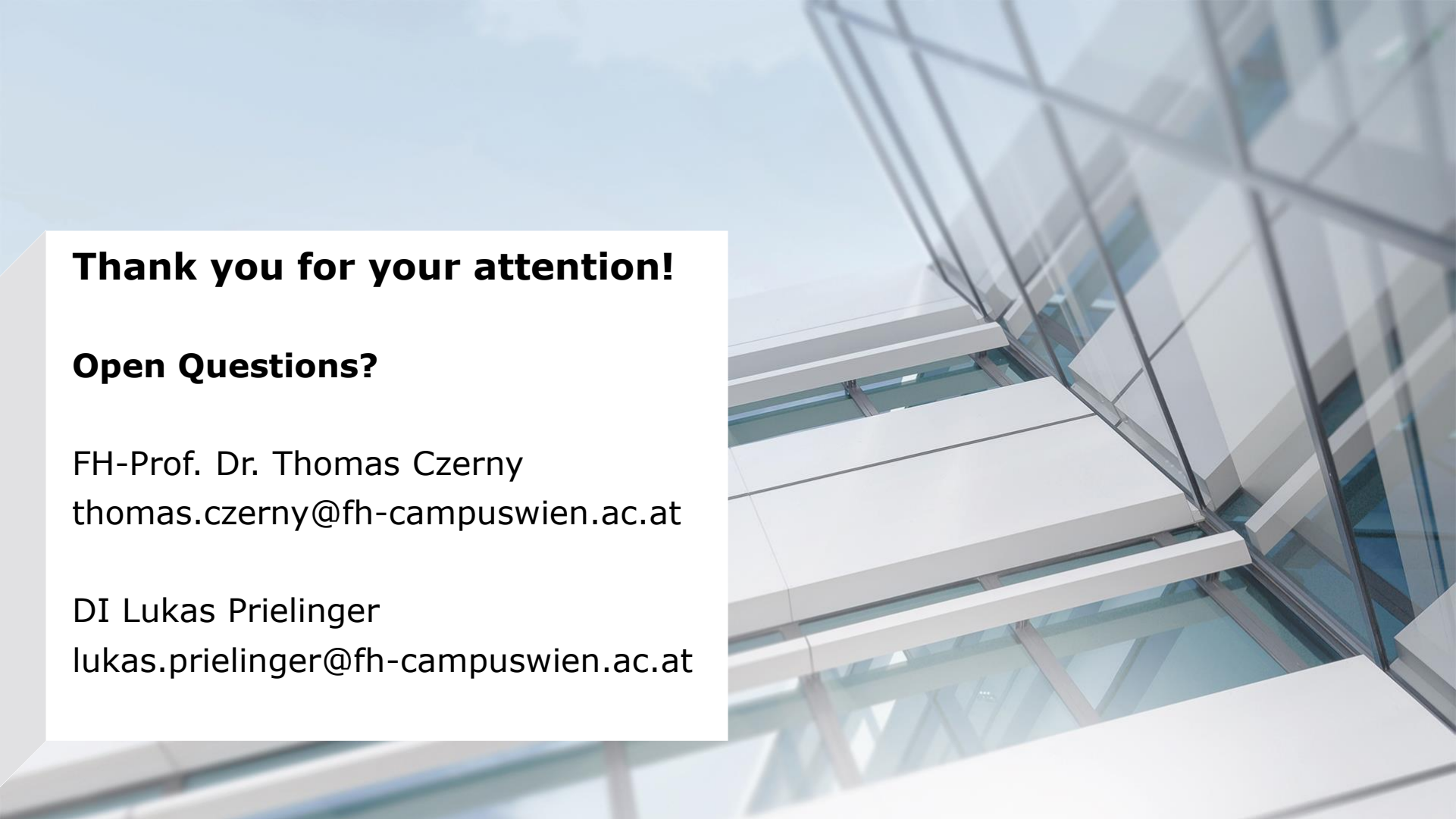
>Extended exposure + further optimizations

>Analysis of pure substances reveals ~70-fold improvement in detection limits

>Analysis of real samples: SenseAmes confirmed Ames MPF data and detected additional positive samples

>Search for follow up projects and commercialization of the SenseAmes

>Poster on SenseAmes at the symposium tomorrow



Thank you for your attention!

Open Questions?

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