Genotoxicity assessment of enniatins and *Alternaria* toxins with the *in vitro* micronucleus assay and the SOS/umu test

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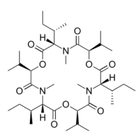
Background

European Partnership for Assessment of Risk from Chemicals (PARC)

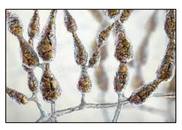
WP5 – Hazard Assessment

T5.1. Toxicity testing addressing data gaps of concern

P5.1.1.a_Y1_Natural toxins

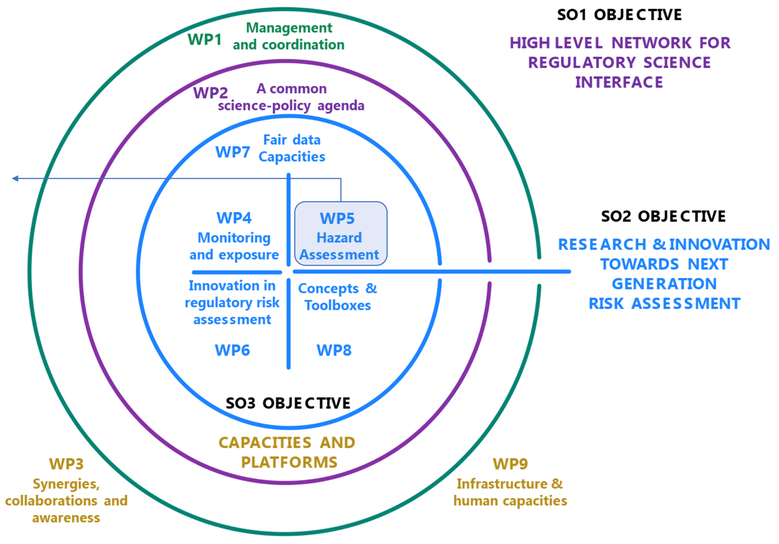


Enniatins



Alternaria toxins

Several endpoints investigated

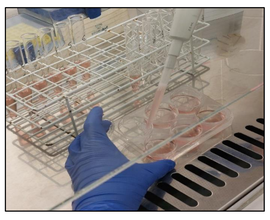


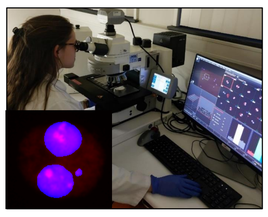
sciensano & Universidad de Navarra involved as partner for the toxicological endpoint **genotoxicity**

Material and methods

***In vitro* micronucleus (MN) assay (OECD TG487)**

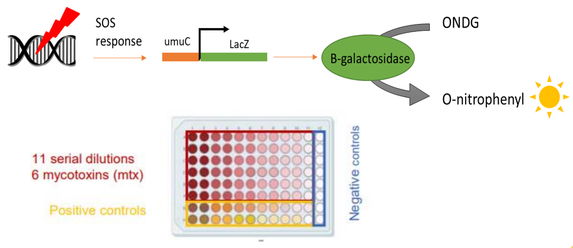
- **Chromosomal aberration** detection
- **Human**-derived **TK6 cells**
- % micronucleated cells scored by **microscope analysis** (Metafer system)
- CBPI (cytokinesis block proliferation index) as indication for cytotoxicity





SOS/umu test (screening for OECD TG471)

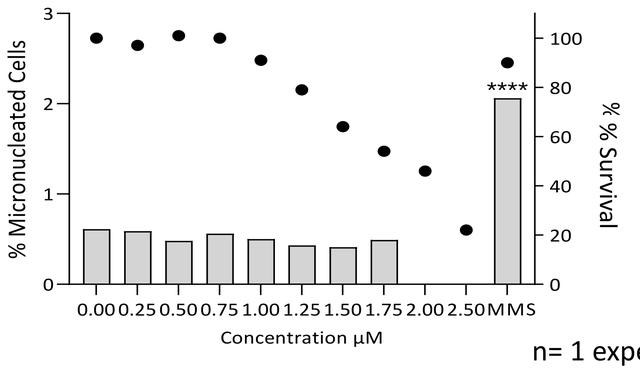
- **Genotoxicity** screening test
- **Bacterial strain TA1535**
- DNA damage assessed by **colorimetric reaction** (absorbance 420 nm)
- Cytotoxicity assessed by % survival (absorbance 600nm)



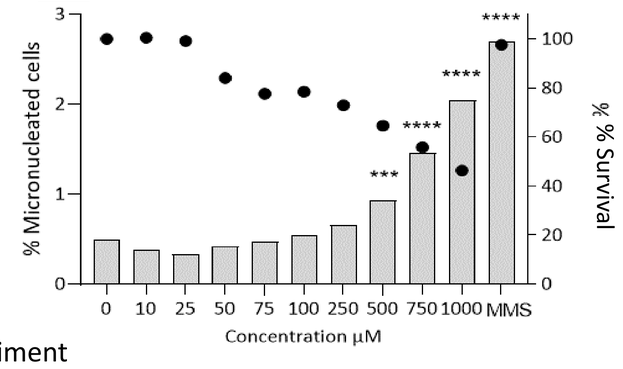
Results

***In vitro* MN test**
24h exposure without S9

A Enniatin B 24h –S9



B Tenuazonic acid 24h –S9



n= 1 experiment

* p < 0.05 ; **: p < 0.01 ; ***: p < 0.001 ; ****: p < 0.0001

Not genotoxic up to cytotoxic concentration

Genotoxic from 500 µM

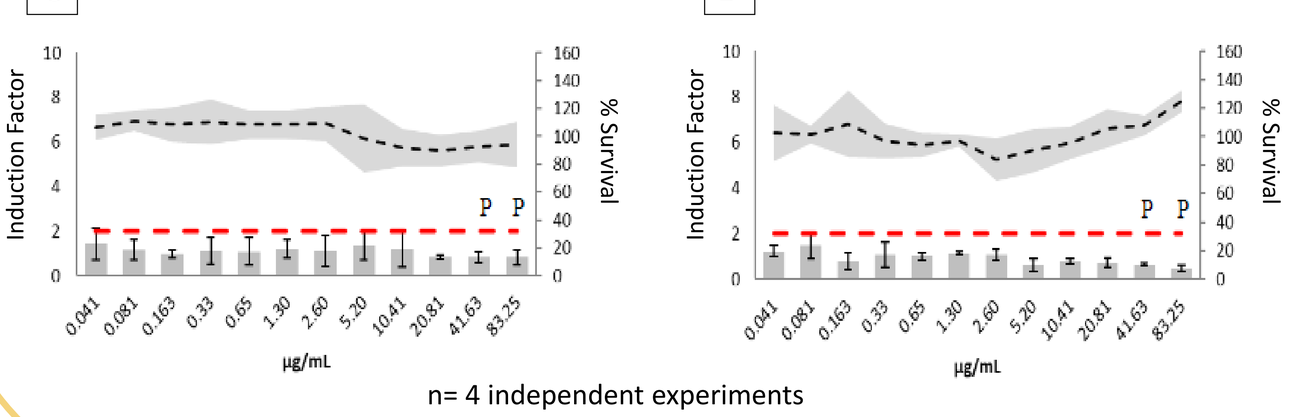
	***In vitro* MN test**			**SOS/umu test**	
Mycotoxins	**24h without S9**	**3h with S9**	**3h without S9**	**4h without S9**	**4h with S9**
Enniatin B	- [A]	-	-	-	-
Enniatin B1	-	-	-	-	-
Enniatin A	-	-	-	- [C]	- [D]
Enniatin A1	-	-	-	-	-
Beauvericin	-	-	-	-	-
Teneazonic acid	+ [B]	-	-	⌛	⌛
Alternariol monomethyl ether	+	⌛	⌛	⌛	⌛
Tentoxin	⌛	⌛	⌛	⌛	⌛
Altertoxin I	+	⌛	⌛	⌛	⌛
Altenuene	⌛	⌛	⌛	⌛	⌛
Alternariol	+*	⌛	⌛	⌛	⌛

**Tests performed with a different batch than the one used in PARC*

SOS/umu test
Without and with S9

C Enniatin A 4h –S9

D Enniatin A 4h +S9



n= 4 independent experiments

P : precipitation

Conclusion

All enniatins tested so far were negative in the *in vitro* MN test and in the SOS/umu test, both in absence and in presence of metabolic activation. In contrast, **tenuazonic acid, alternariol, alternariol monomethyl ether and altertoxin-I induced a clear positive effect in the *in vitro* MN test after 24h in absence of metabolic activation.**

Next steps

- Perform the *in vitro* micronucleus and SOS/umu tests with the mycotoxins that have not yet been tested
- Share the results with PARC partners and compare with the genotoxicity results obtained in other assays (i.e. AMES test)

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Health Food Chain Safety Environment

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