sciensano

healthy all life long

Towards a harmonized strategy to evaluate combined effects of genotoxicants

J. Sanders, G. Johnson, R. Anthonissen, P. Becker, E. Tangni, J. Masquelier, T. Vanhaecke and B. Mertens

Towards characterization and genotoxicity assessment of mycotoxins in food and feed

Towards a harmonized strategy for the genotoxicity assessment of mixtures

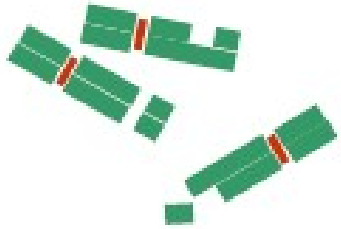
.be

Genotoxicity

Gene mutations

GGAAGTCTA
CGT TCAGAT
↓
GGAAATCTA
CGT TTAGAT

Structural chromosome aberrations



Numerical chromosome aberrations

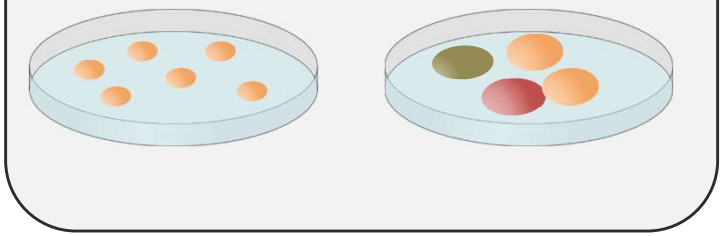
BATTERY OF DIFFERENT *IN VITRO* (AND *IN VIVO*) TESTS NEEDED

Genotoxicity testing

GENE MUTATIONS

Ames test

MLA/TK/HPRT

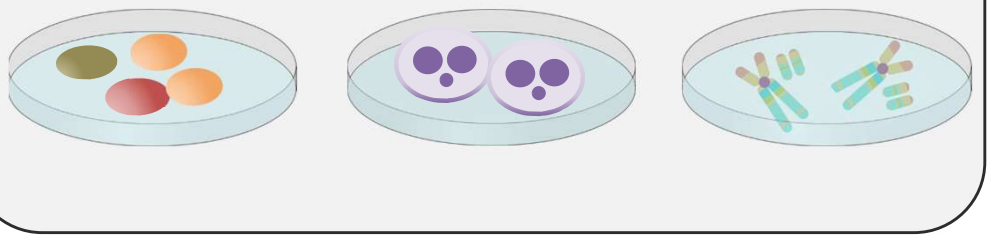


STRUCTURAL and/or NUMERICAL CHROMOSOME ABERRATIONS

MLA/TK

Micronucleus test

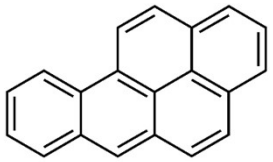
Chromosome aberration test



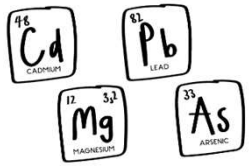
Follow-up testing in adequate *in vivo* test (if allowed)

Focus on assessment of individual compounds and NOT on mixtures

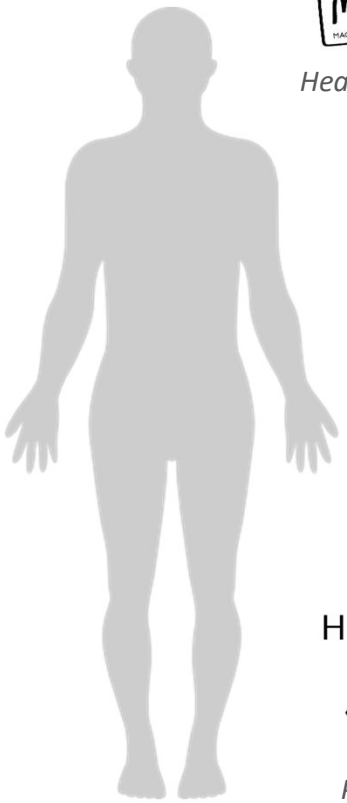
Mixtures of genotoxicants

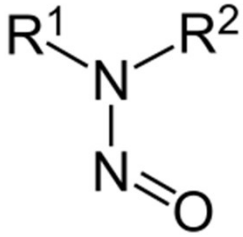


Polyclic aromatic hydrocarbons



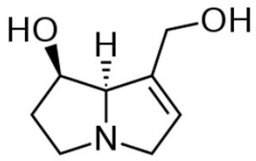
Heavy metals





Nitrosamines

Mycotoxins



Pyrrolizidine alkaloids

EFSA opinion on genotoxicity assessment

STATEMENT

EFSA Journal

ADOPTED: 22 November 2018

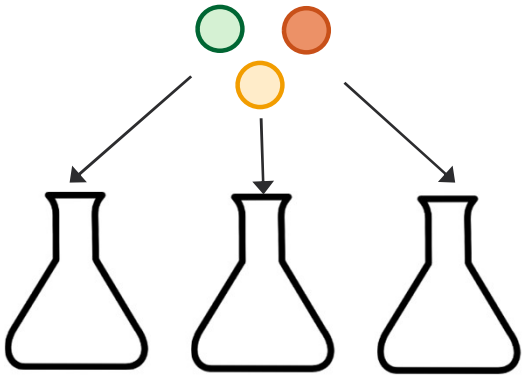
doi: 10.2903/j.efsa.2019.5519

Genotoxicity assessment of chemical mixtures

EFSA Scientific Committee,
Simon More, Vasileios Bampidis, Diane Benford, Jos Boesten, Claude Bragard, Thorhallur Halldorsson, Antonio Hernandez-Jerez, Susanne Hougaard-Bennekou, Kostas Koutsoumanis, Hanspeter Naegeli, Søren Saxmose Nielsen, Dieter Schrenk, Vittorio Silano, Dominique Turck, Maged Younes, Gabriele Aquilina, Riccardo Crebelli, Rainer Gürtler, Karen Ildico Hirsch-Ernst, Pasquale Mosesso, Elsa Nielsen, Roland Solecki, Maria Carfì, Carla Martino, Daniela Maurici, Juan Parra Morte and Josef Schlatter

EFSA opinion on genotoxicity assessment

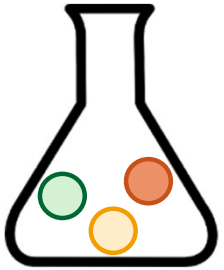
Genotoxicity dives risk assessment (health-based guidance value vs margin of exposure approach)



Compound-based approach

Chemically fully defined mixtures

Versus

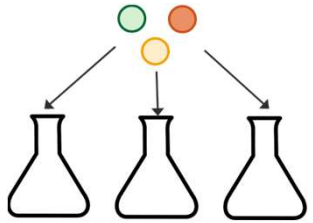


Whole mixture approach

Mixtures containing a substantial fraction of unidentified components

Chemical characterization and demonstration of stability of mixtures is required

Chemically fully-defined mixtures

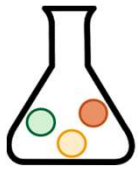


1. If one or more compounds are **genotoxic *in vivo***, the mixture raises concern for genotoxicity

2. If none of the compounds raises a concern with respect to genotoxicity, the mixture is also considered of **no genotoxic concern**

3. If there are compounds with **a potential concern for genotoxicity** but for which the data available are not sufficient to conclude on genotoxicity, additional data are needed to complete an assessment

Mixtures containing a substantial fraction of unidentified components



1. Chemically identified substances need to be assessed **individually for their potential genotoxicity**, using all available information

2. If none of the identified compounds raises a concern with respect to genotoxicity, the **unidentified fraction should also be evaluated**

3. Experimental testing of the unidentified fraction should be considered as the first option or, if this is not feasible and a scientific justification is provided, **testing of the whole mixture** should be undertaken

Focus on hazard identification, no (quantitative) evaluation of the combined effects!

Type of combined effects

Non-genotoxic endpoints:

Additivity	effect (A & B) = A + B
Synergy	effect (A & B) > A + B
Antagonism	effect (A & B) < A + B

Does the principle of additivity also apply to genotoxic compounds?

Polycyclic aromatic hydrocarbons

Mutation Research/Genetic Toxicology and Environmental Mutagenesis
Volume 827, March 2018, Pages 9-18

Genotoxicity evaluation of multi-component mixtures of polyaromatic hydrocarbons (PAHs), arsenic, cadmium, and lead using flow cytometry based micronucleus test in HepG2 cells

Sasikumar Muthusamy [a b], Cheng Peng [a b], Jack C. Ng [a b]

Mutation Research 515 (2002) 85–98
Genetic Toxicology and Environmental Mutagenesis
www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

The genotoxicity of priority polycyclic aromatic hydrocarbons in complex mixtures

Paul A. White*
NRC Research Associate, Atlantic Ecology Division, United States Environmental Protection Agency, 27 Tarzwell Drive, Narragansett, RI 02882, USA

Carcinogenesis vol.28 no.12 pp.2632–2640, 2007
doi:10.1093/carcin/bgm182
Advance Access publication August 8, 2007

Binary PAH mixtures cause additive or antagonistic effects on gene expression but synergistic effects on DNA adduct formation

toxics
MDPI

Article

In Vitro Genotoxicity Evaluation of PAHs in Mixtures Using Experimental Design

Rebecca Castel [1,2], Virginie Tassistro [1], Magalie Claeys-Bruno [1], Laure Malleret [2] and Thierry Orsière [1,*]

Environment International 166 (2022) 107345
Contents lists available at ScienceDirect
Environment International
journal homepage: www.elsevier.com/locate/envint

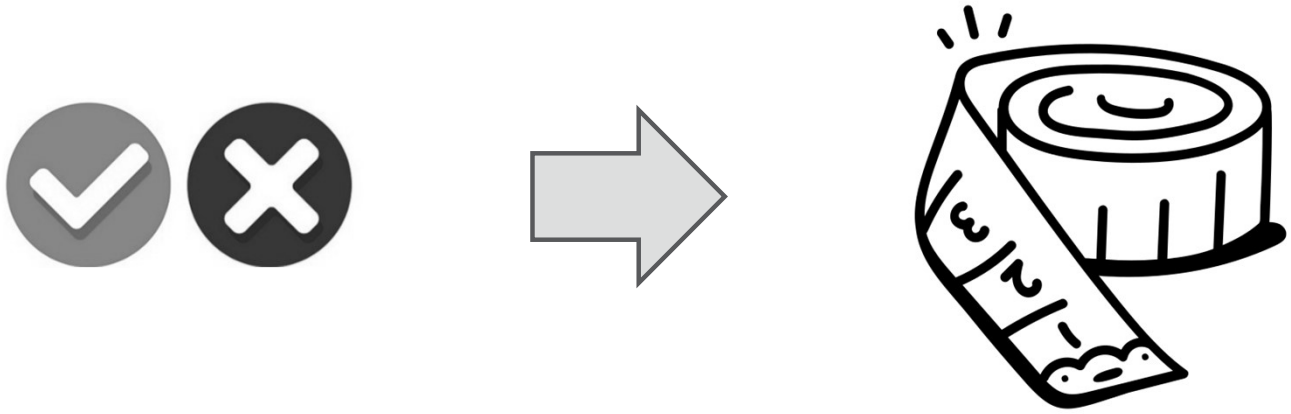
Full length article

Determination of whole mixture-based potency factors for cancer risk assessment of complex environmental mixtures by *in vitro* testing of standard reference materials

Marcos Felipe de Oliveira Galvão [a], Ioannis Sadiktsis [b], Tiago Marques Pedro [a], Kristian Dreij [a,*]

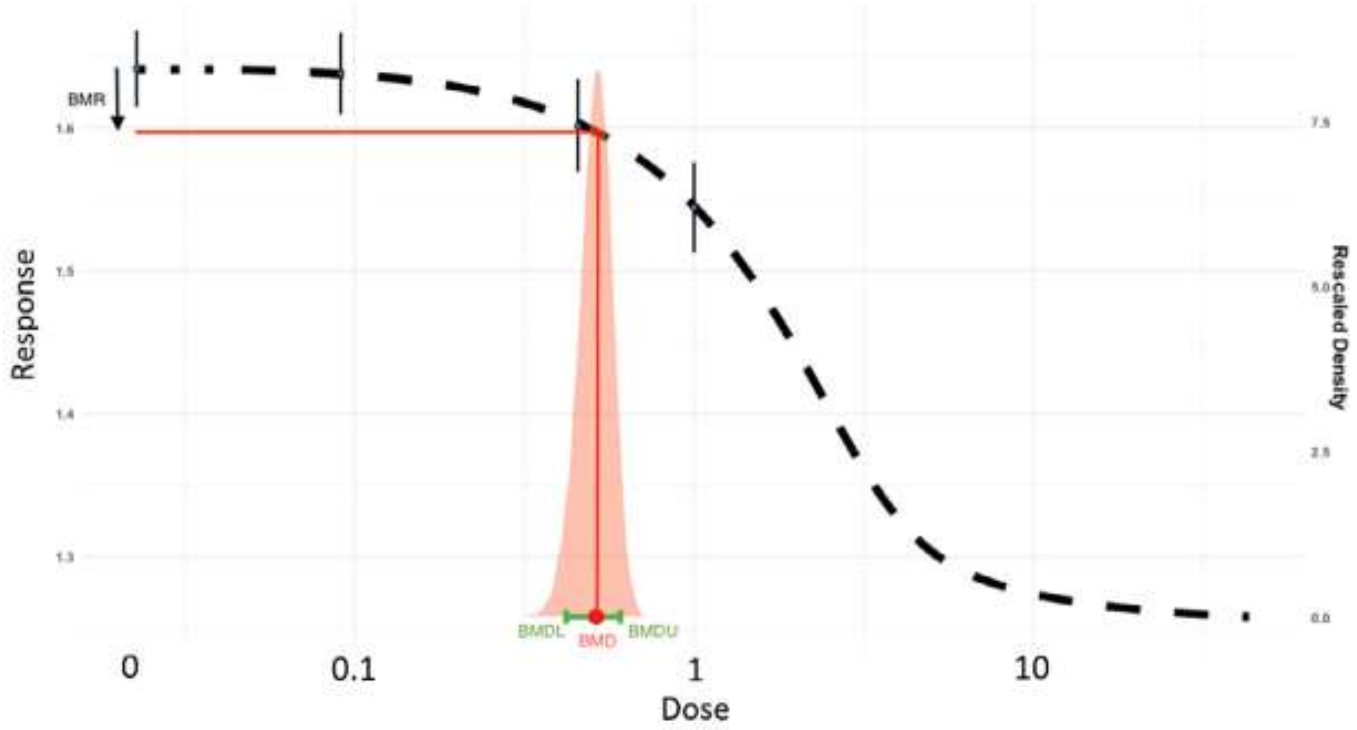
Different outcomes (additive, synergistic and/or antagonistic effects)

Limitations of current genotoxicity assessment



Quantitative assessment of genotoxicity data is needed to evaluate combined effects!

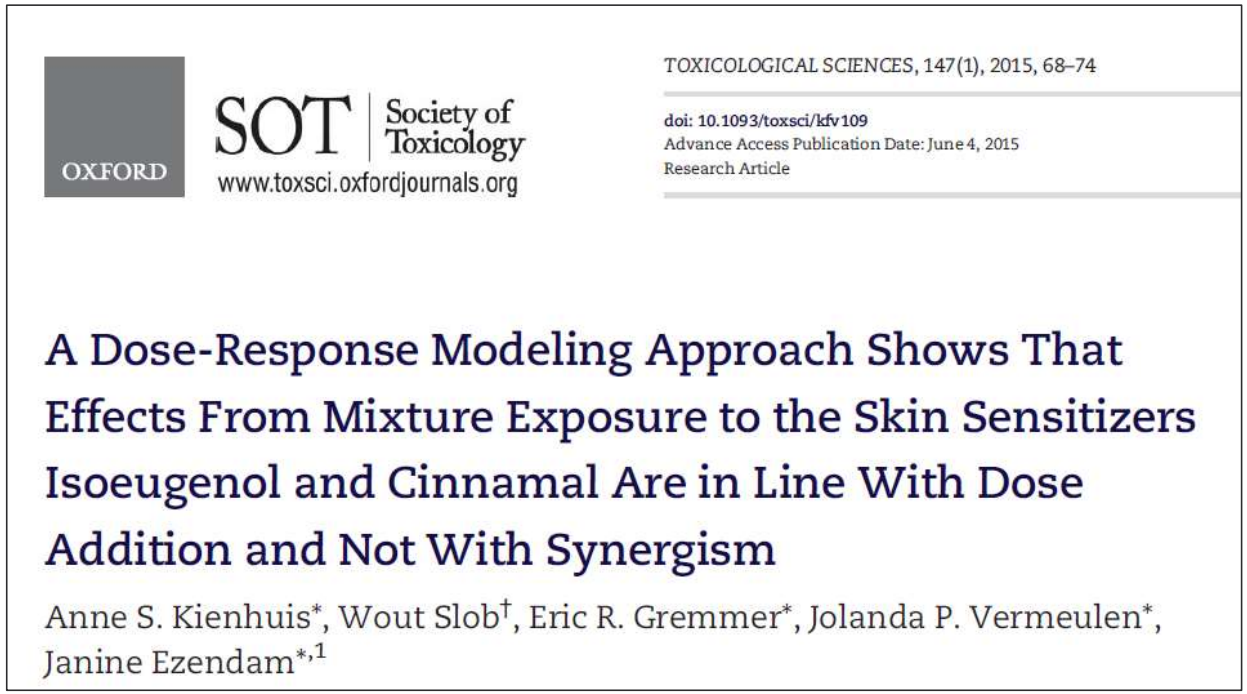
Benchmark dose (BMD) approach



Co-variate approach allows to compare potencies of different compounds

BMD lower limit (BMDLx): dose below which the change in response is likely to be smaller than x% where the term 'likely' is defined by the statistical credible level, usually 95%-level

BMD-based approach for skin sensitizers



OXFORD

SOT | Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 147(1), 2015, 68–74

doi: 10.1093/toxsci/kfv109
Advance Access Publication Date: June 4, 2015
Research Article

A Dose-Response Modeling Approach Shows That Effects From Mixture Exposure to the Skin Sensitizers Isoeugenol and Cinnamal Are in Line With Dose Addition and Not With Synergism

Anne S. Kienhuis*, Wout Slob†, Eric R. Gremmer*, Jolanda P. Vermeulen*, Janine Ezendam*,1

Underlying principle: To assess whether or not responses from mixtures of sensitizers can be predicted from the dose-response information obtained from individual chemicals using dose addition

BMD-based approach for skin sensitizers

1. Determine the **dose-response** of chemical A and that of chemical B; Establish if both dose-responses are approximately parallel on log-dose scale *(response = 3 immunological parameters)*

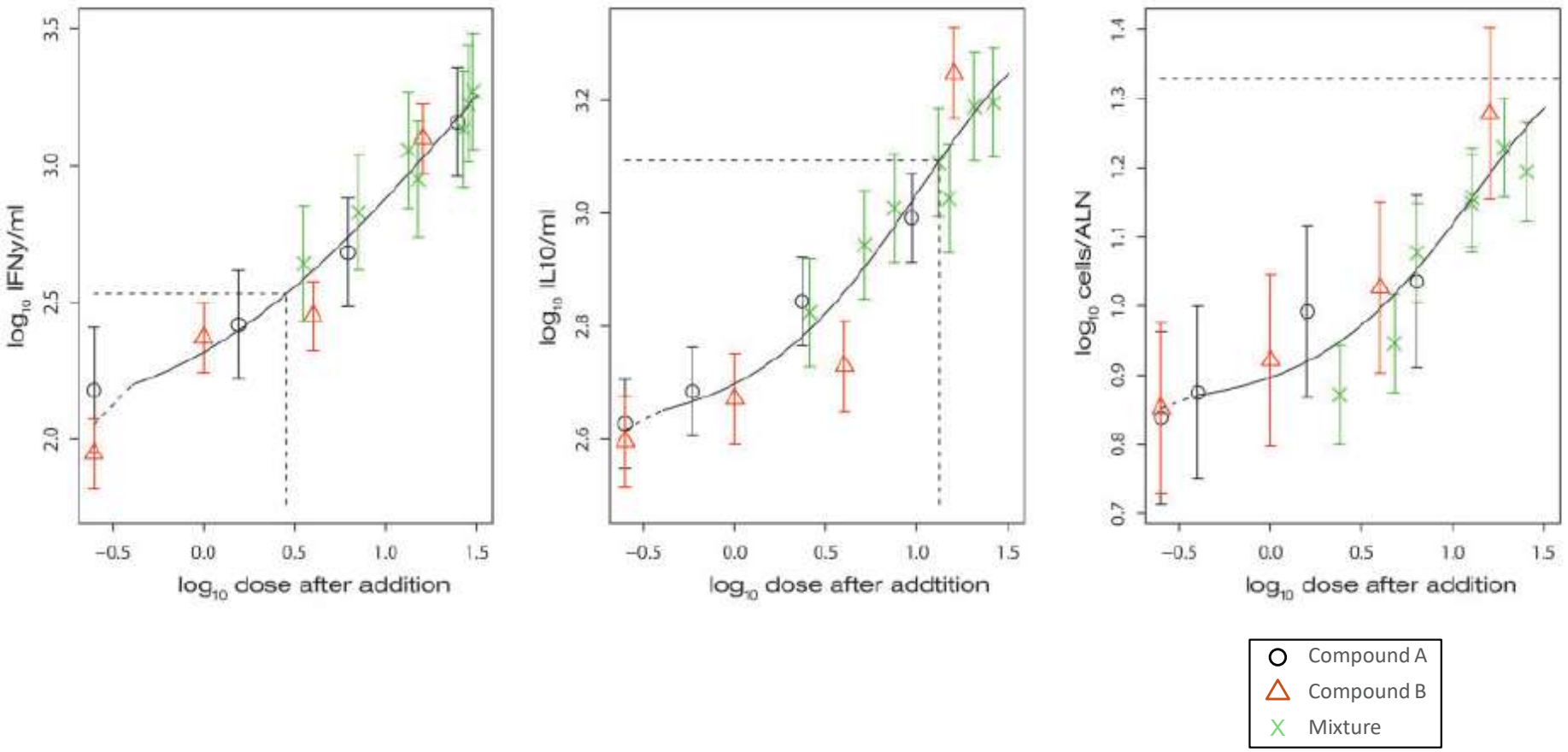
2. If it can be assumed that the dose-responses of A and B are parallel on log-scale, **calculate the relative potency factor (RPF)**; note that log(RPF) is the horizontal distance between both dose-response curves on log-dose scale

3. Calculate the dose of chemical A that is **equipotent** to dB, by multiplying dB by the RPF

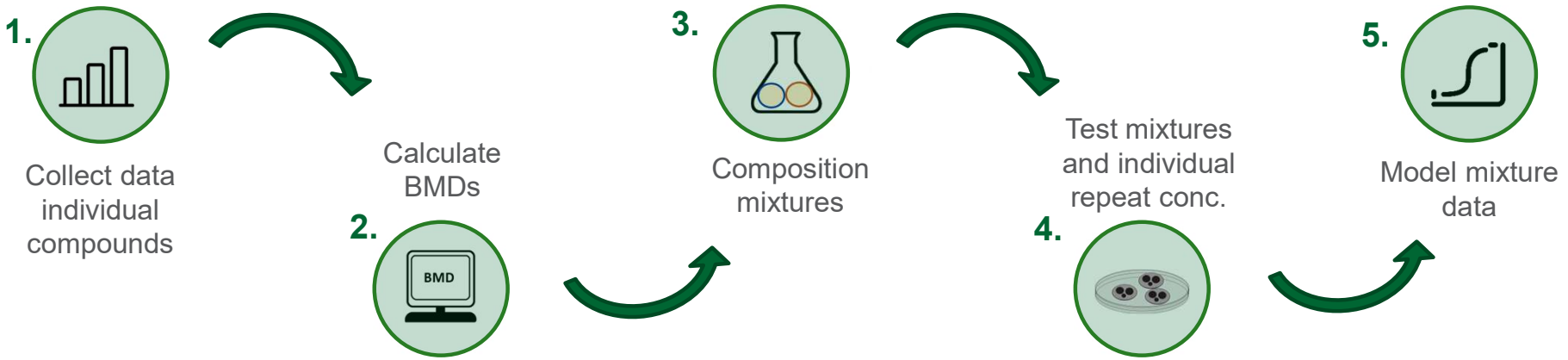
4. Add the original dA to the dose calculate in step 3

5. **Read of the response at the added dose** using the dose-response of chemical A

BMD-based approach for skin sensitizers



BMD-based approach for genotoxicity testing



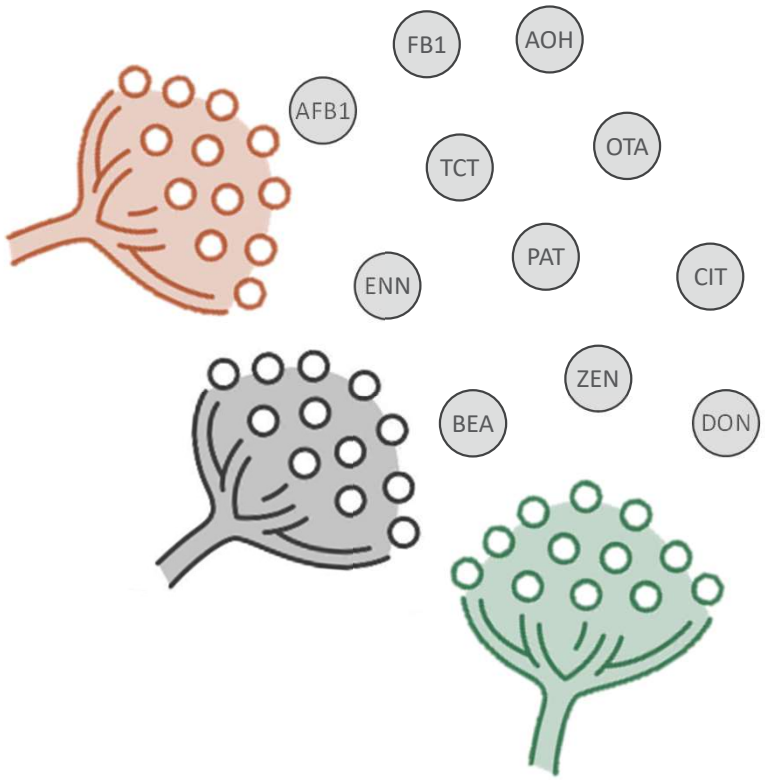
Genotoxicity data (in vitro MN data)

Derive estimated RPE

Prepare mixtures inducing several levels of genotoxicity (BMR25, BMR50, BMR100, BMR150 & BMR 200) in different ratio's (1:1; 3:1 and 1:3)

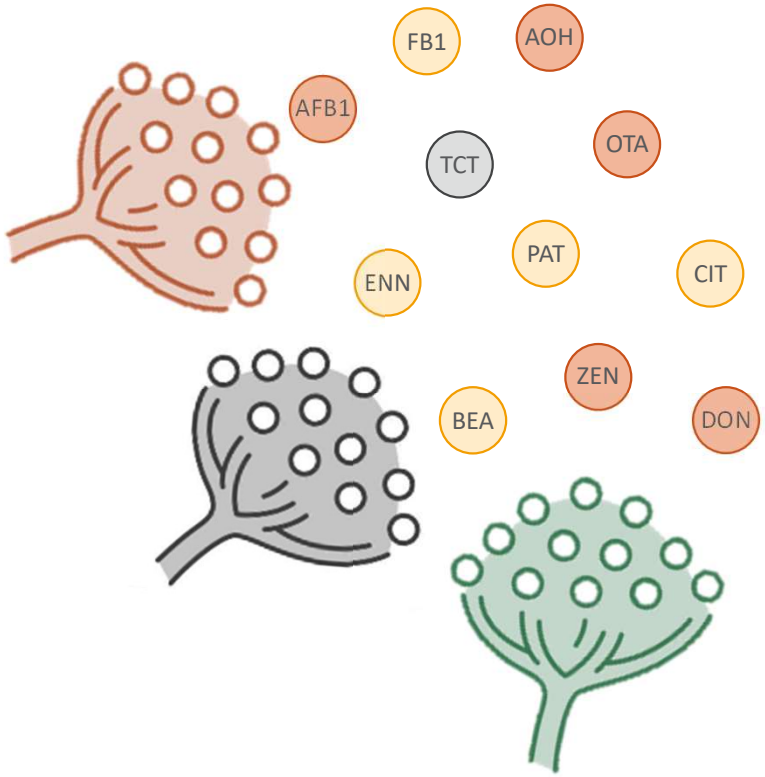
PROAST package in R: dose-addition model 15

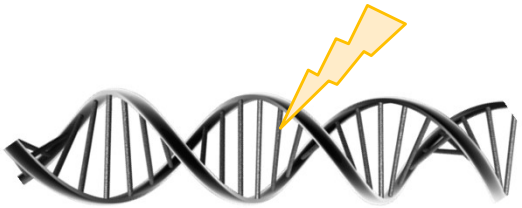
Mycotoxins



AFB1: aflatoxin B1; AOH: alternariol; BEA: beauvericin; CIT: citrinin; DON: deoxynivalenol; ENN: enniatins; FB1: fumonisin B1; OTA: ochratoxin A; PAT: patuline; TCT: trichothecenes; ZEN: zearalenone

Mycotoxins

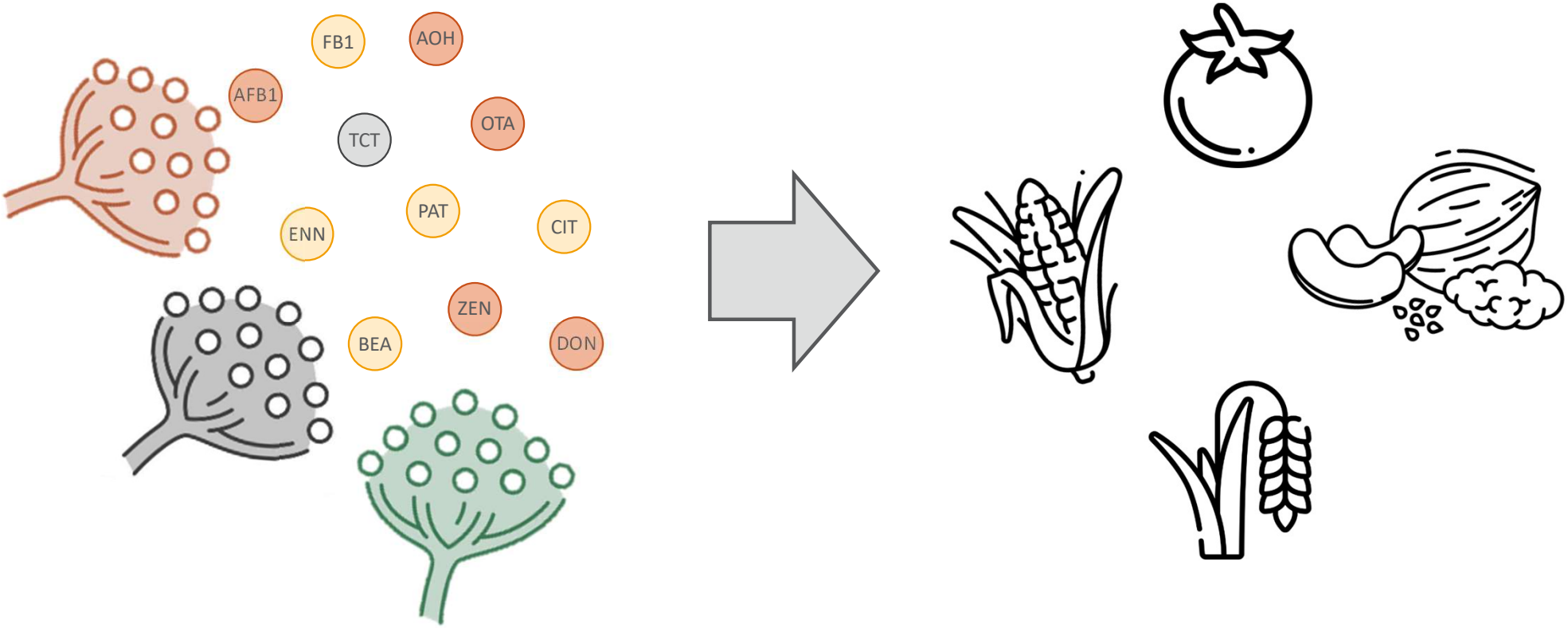




Several mycotoxins are (suspected) genotoxins

AFB1: aflatoxin B1; AOH: alternariol; BEA: beauvericin; CIT: citrinin; DON: deoxynivalenol; ENN: enniatins; FB1: fumonisin B1; OTA: ochratoxin A; PAT: patuline; TCT: trichothecenes; ZEN: zearalenone

Mycotoxins



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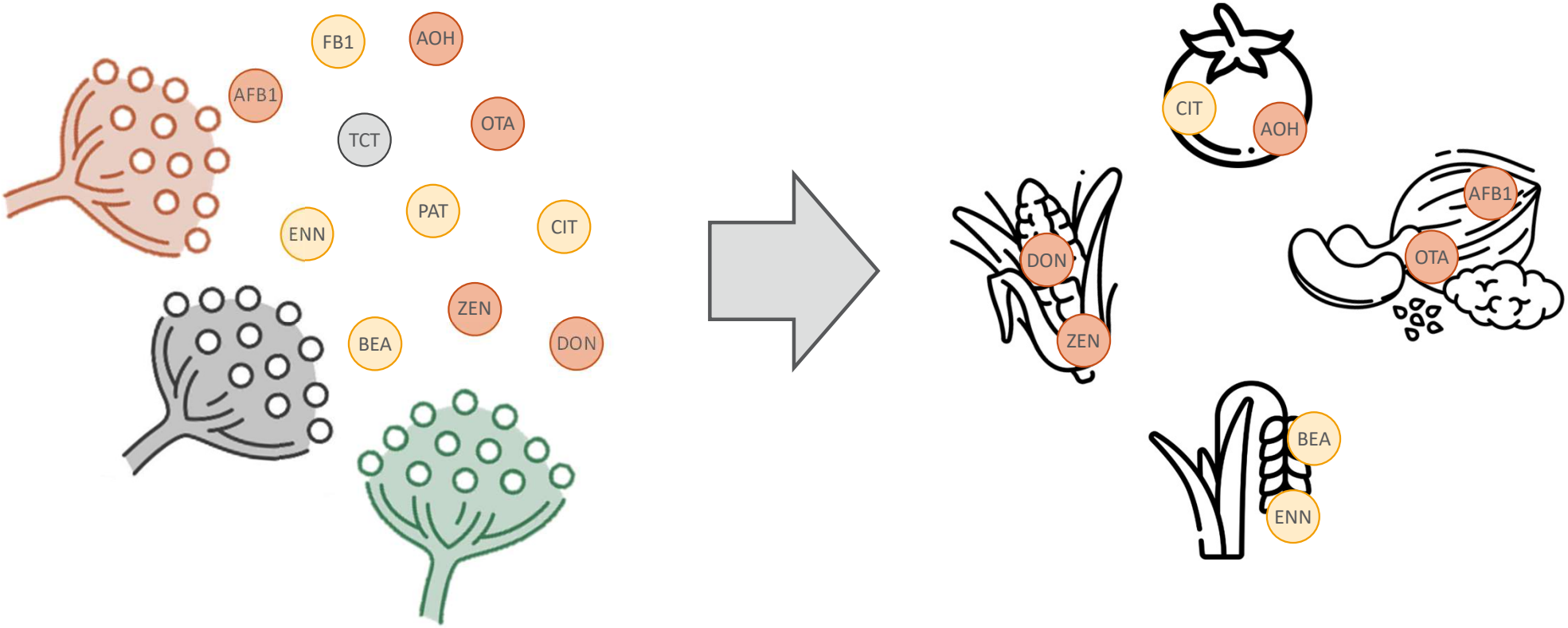
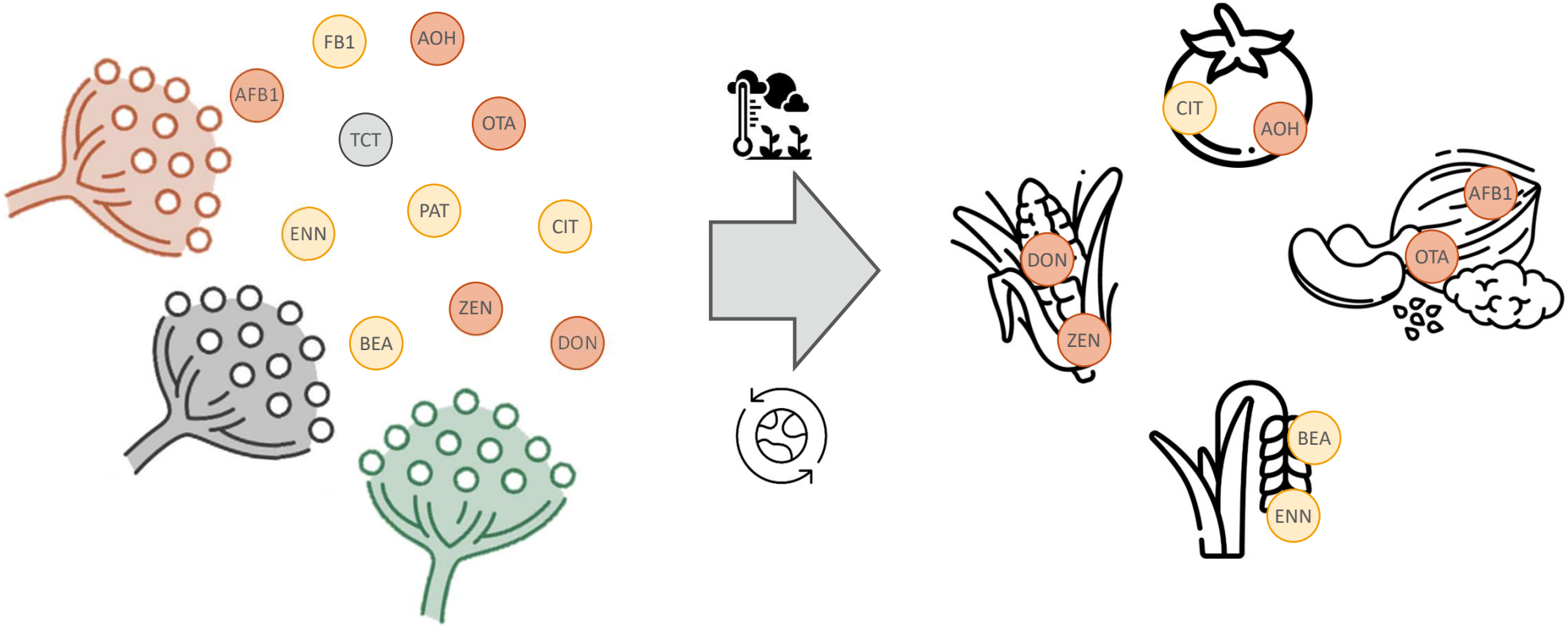
Mycotoxins



AFB1: aflatoxin B1; AOH: alternariol; BEA: beauvericin; CIT: citrinin; DON: deoxynivalenol; ENN: enniatins; FB1: fumonisin B1; OTA: ochratoxin A; PAT: patuline; TCT: trichothecenes; ZEN: zearalenone

Mycotoxins



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MYCX-IT project

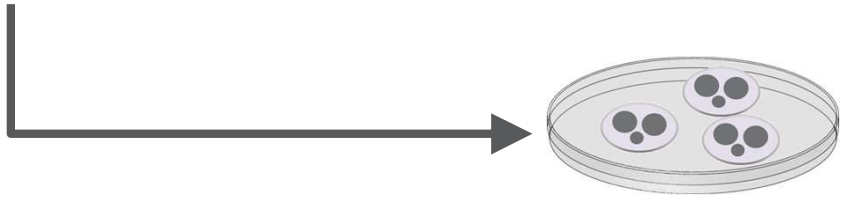
VRIJE UNIVERSITEIT BRUSSEL

Swansea University Prifysgol Abertawe

To **quantitatively** assess the **genetic damage** induced by exposure to relevant mixtures of **co-occurring mycotoxins**

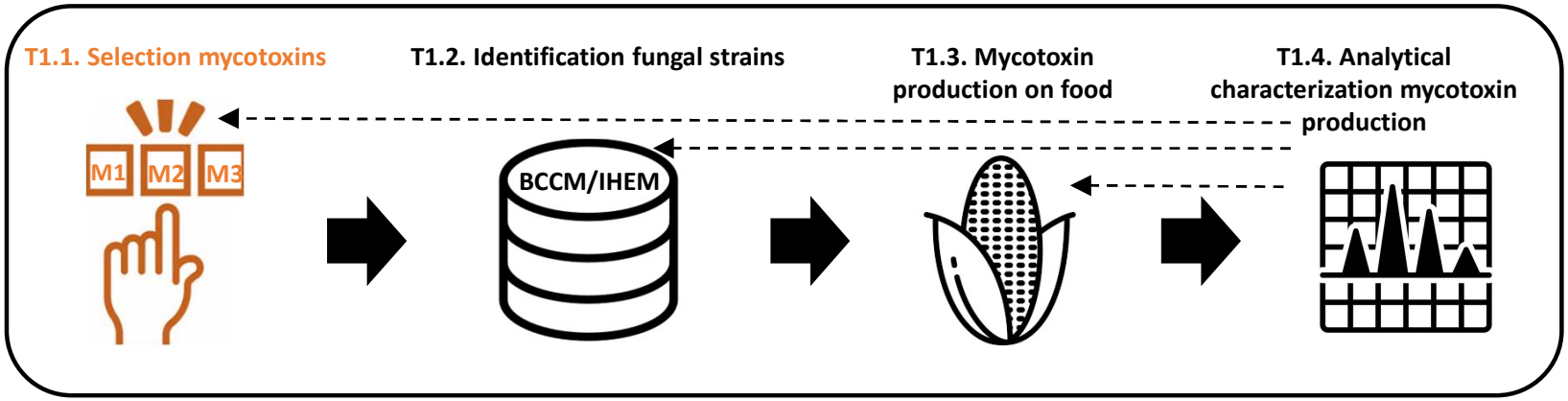
Two types of mixtures:

1. Self-made mixtures of co-occurring mycotoxins
2. Natural extracts produced by fungal strains



J. Sanders

Characterization of fungal strains

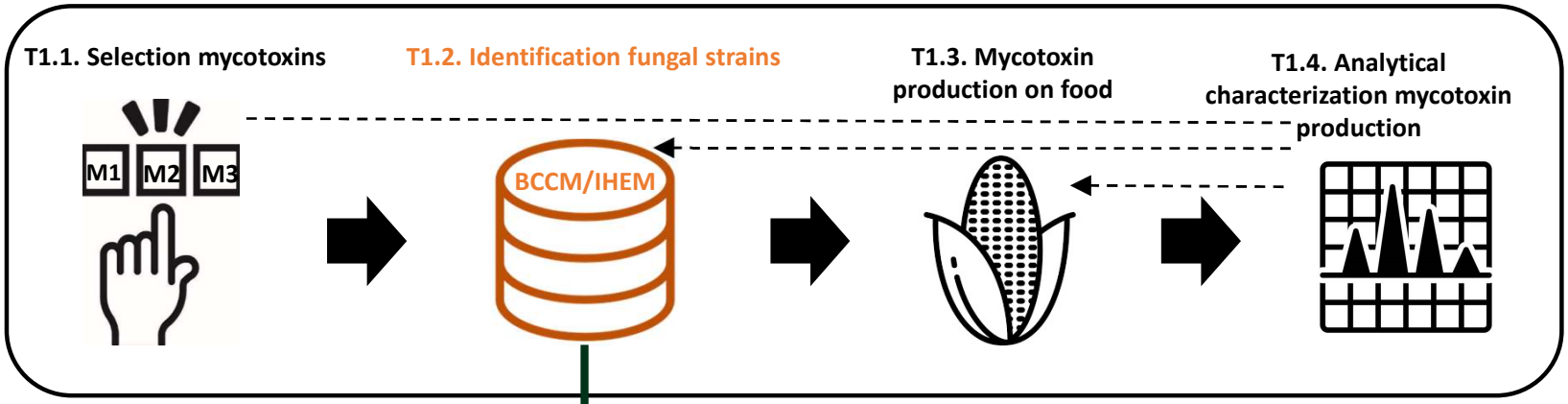


Three criteria considered in the selection process:

- Co-occurrence in maize
- Genotoxic potential and mode of action *(clastogenic and/or aneugenic effect)*
- Regulatory status

Selected mycotoxins
Aflatoxin B1 (AFB1)
Alternariol (AOH)
Beauvericin (BEA)
Deoxynivalenol (DON)
Fumonisin B1 (FB1)
Zearalenone (ZEN)

Characterization of fungal strains



Database

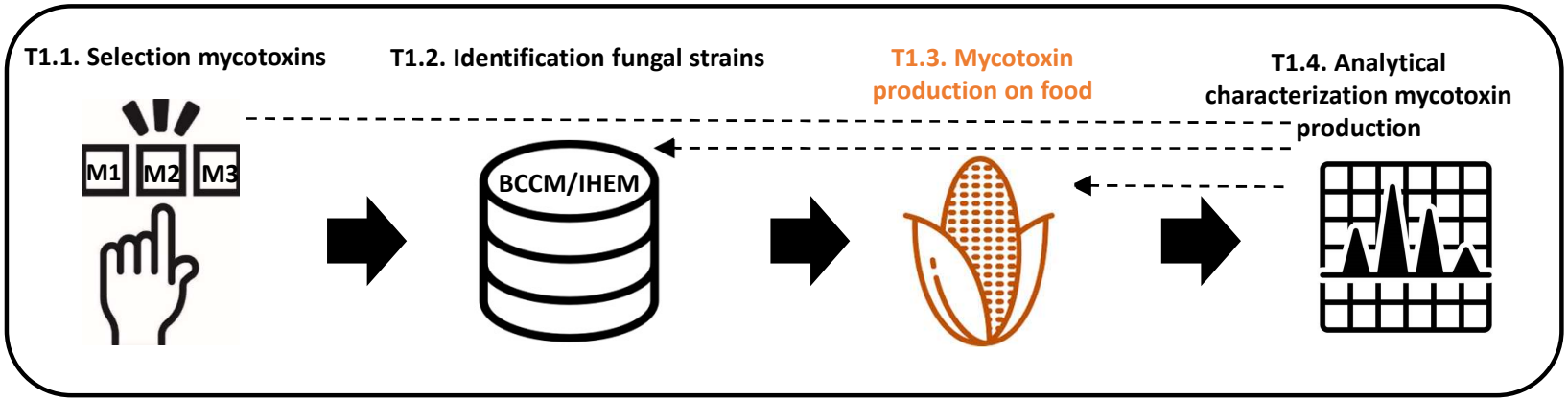
EXL

Preferably fungal strains isolated from food

Information on:

- Food substrate
- Occurrence in maize
- Production of mycotoxins

Characterization of fungal strains

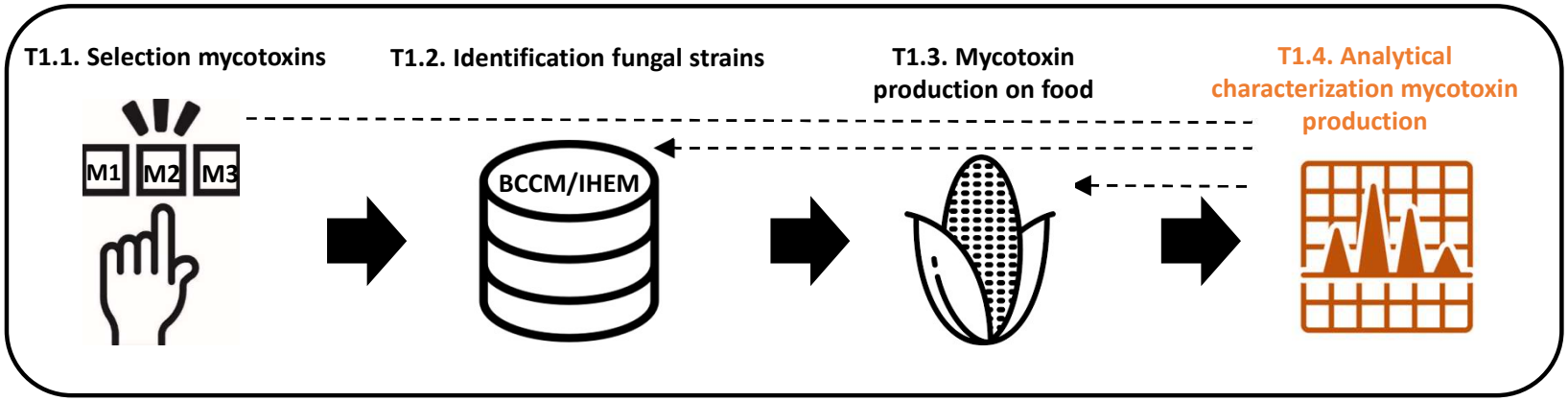


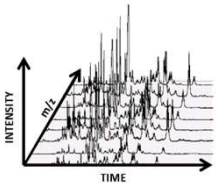
Control

A. Aborescens -1367

A. Aborescens -10114

Characterization of fungal strains





Development, optimisation and validation of a **multimycotoxin LC-MS method**

Fully validated for **22 mycotoxins**

AFB1	**DON**	OTA
AFB2	3AcDON	HT2
AFG1	15AcDON	**ZEN**
AFG2	ENN A	CIT
FB1	ENN A1	**AOH**
FB2	ENN B	TEN
FB3	ENN B1	**BEA**

+ T2 → Only qualitative detection

Concentration mg/kg **individual strains**

		Sample ID	AFB1	AFB2	AFG1	AFG2	OTA	FB1	FB2	FB3	T2	HT2	DON	ZEN	CIT	AOH	TEN	/15AcDO	BEA	ENN A	ENN A1	ENN B	ENN B1	NIV	DON3Glu
Control	1							0,16	0,11	0,02			0,28	0,14				0,07							
	2							0,05	0,03	0,01			0,17					0,05							
	3							0,03	0,02	0,01			0,02	0,03				0,05							
A. aborescens 1367	1															2606,98	0,97								
	2															2216,32	1,09								
A. aborescens 10114	1															1536,86	15,71								
	2															2207,67	17,03								
A. parasiticus 4387	1		102,85	3,61418	28,074	1,32188																			
	2		95,0954	3,53626	26,5415	1,23969																			
A. parasiticus 4383	1		292,29	17,99	170,11	12,75																			
	2		276,11	15,06	141,86	11,24																			
A. flavus 4388	1		0,01		0,01			0,21	0,16	0,13															
	2		0,02		0,01			0,1	0,1	0,11															
F. poae 13813	1																		40,01						
	2																		51,42						
F. culmorum 3322	1													274,98											
	2													166,61											
F. verticillioides 10153	1							409,03	229,01	66,49															
	2							384,69	210,04	73,66															
F. subglutinans 3820	1																								
	2																								
F. cerealis 14083	1													379,33											
	2													553,26											
F. oxysporum 3798	1																		9,26						
	2																		10,44						
F. oxysporum 13830	1																		12,81						
	2																		13,39						
F. avenaceum 14084	1																				1,32	141,73	20,43		
	2																				1,11	113,5	16,93		
F. verticillioides 23528	1							1865,16	1419,05	586,22															
	2							1991,61	1458,62	622,64															
F. graminearum 2995	1												118,07	48,83				40,81							
	2												127,78	81,19				46,47							
F. poae 15929	1											294,62							34,98						
	2											649,64							63,26						
F. graminearum 53186	1												465,81	1302,67				164,29							
	2												303,33	1499,74				150,77							
F. proliferatum 10152	1							720,83	115,68	64,79									4,83						
	2							584,32	98,92	49,84									4,46						
A. nomius 2262	1		0,08		0,07			0,25	0,18																
	2		0,08		0,09			0,87	0,62																
A. flavus 23903	1		4	0,13																					
	2		5,08	0,12																					
A. alternata 3121	1															145,87									
	2															118,02									

Concentration mg/kg **individual strains**

		Sample ID	AFB1	AFB2	AFG1	AFG2	OTA	FB1	FB2	FB3	T2	HT2	DON	ZEN	CIT	AOH	TEN	/15AcDON	BEA	ENN A	ENN A1	ENN B	ENN B1	NIV	DON3Glu
Control	1							0,16	0,11	0,02			0,28	0,14				0,07							
	2							0,05	0,03	0,01			0,17					0,05							
	3							0,03	0,02	0,01			0,02	0,03				0,05							
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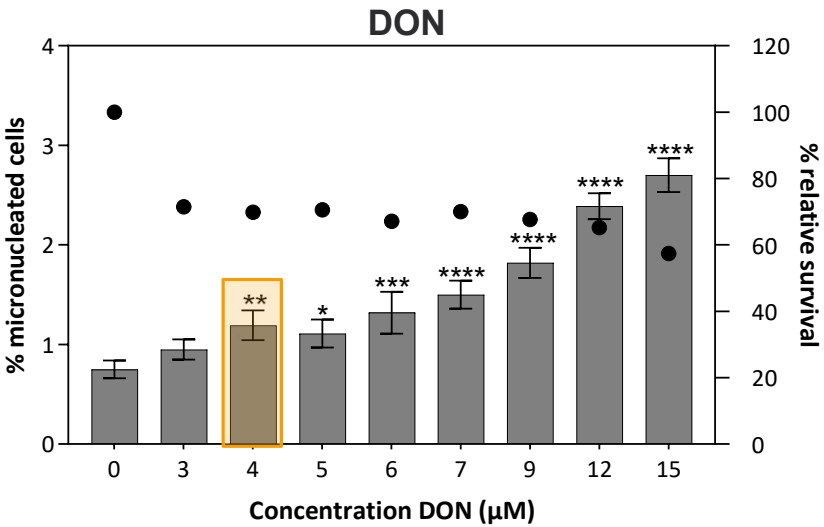
A. parasiticus 4387	1		102,85	3,61418	28,074	1,32188																			
	2		95,0954	3,53626	26,5415	1,23969																			
A. parasiticus 4383	1		292,29	17,99	170,11	12,75																			
	2		276,11	15,06	141,86	11,24																			
A. flavus 4388	1		0,01		0,01			0,21	0,16	0,13															
	2		0,02		0,01			0,1	0,1	0,11															
F. poae 13813	1																								
	2																		37,42						
F. culmorum 3322	1													274,98											
	2													166,61											
F. verticillioides 10153	1							409,03	229,01	66,49															
	2							384,69	210,04	73,66															
F. subglutinans 3820	1																								
	2																								
F. cerealis 14083	1													379,33											
	2													55											
F. oxysporum 3798	1																		9,26						
	2																		10,44						
F. oxysporum 13830	1																		12,81						
	2																		13,39						
F. avenaceum 14084	1																				1,32	141,73	20,43		
	2																				1,11	113,5	16,93		
F. verticillioides 23528	1							1865		119,05	586,22														
	2							199		158,62	822,64														
F. graminearum 2995	1												118,07	48,83				40,81							
	2												127,78	81,19				46,47							
F. poae 15929	1											294,62							34,98						
	2											649,64							63,26						
F. graminearum 53186	1												465,81	1302,67				164,29							
	2												303,3	[illegible]				[illegible]							
F. proliferatum 10152	1							720,8	115	54,79									4,83						
	2							584	9	49,84									[illegible]						
A. nomius 2262	1		0,08		0,07			0		18															
	2		0,08		0,09			0																	
A. flavus 23903	1		4	0,13																					
	2		5,08	0,12																					
A. alternata 3121	1															145,87									
	2															118,02									

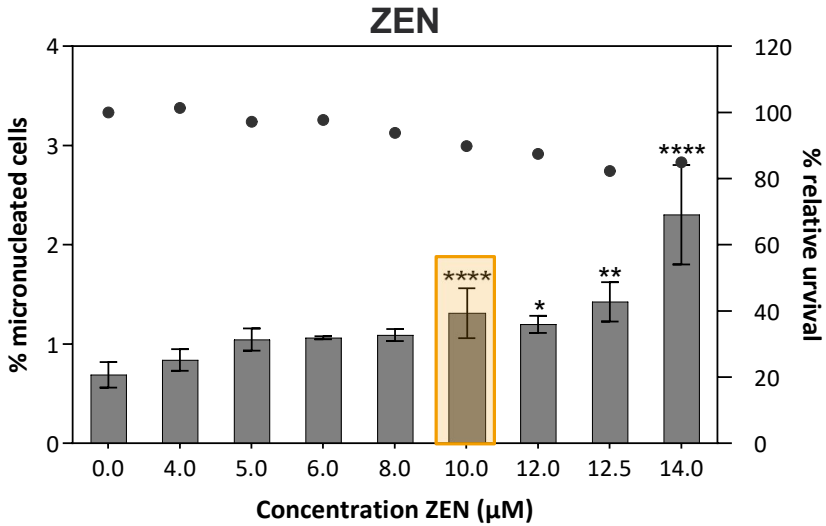
Selection of mycotoxins for mixture testing to evaluate combined genotoxic effects

Case studies with known genotoxicants
(presentation J. Sanders)

Individual genotoxicity data

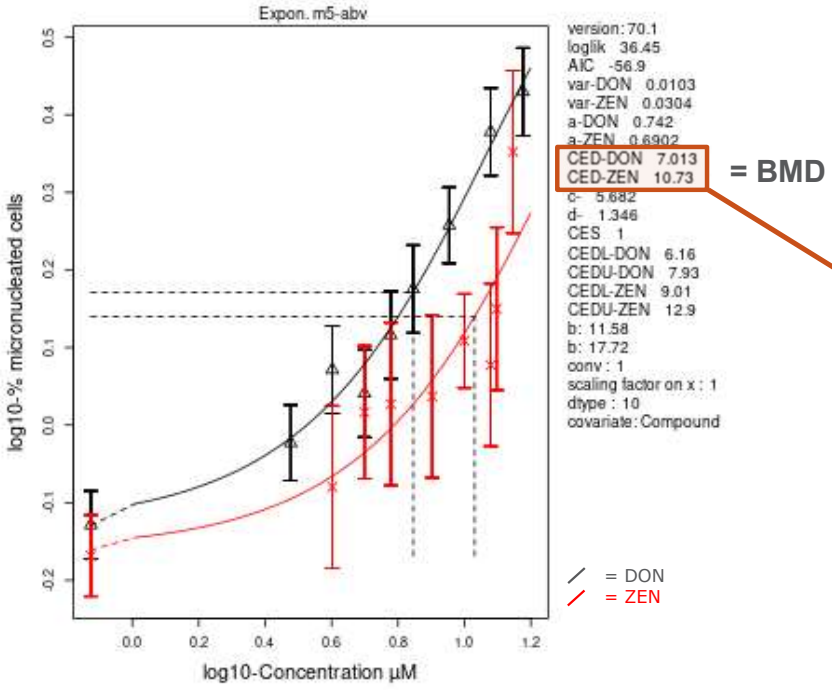
In vitro MN test in TK cells without S9 (n ≥ 3)





BMD co-variate approach

BMR 100%



= BMD

Composition mixtures – BMR 100%

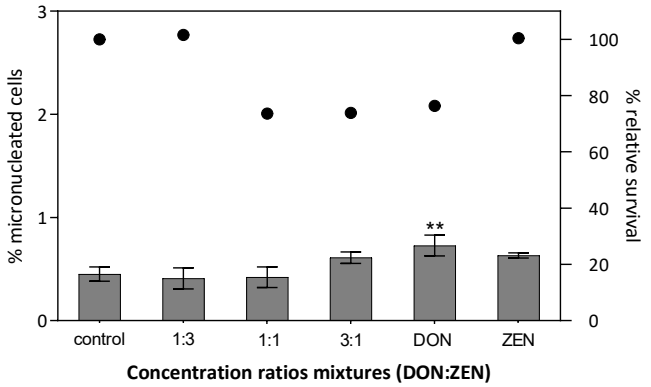
Ratio	Concentration (µM)	
	DON	ZEN
1:3	1.7	8.0
1:1	3.5	5.4
3:1	5.3	2.7
1:0	7.0	0
0:1	0	10.7

Approximate RPF_{zen} = 7.013/10,73 = 0,655

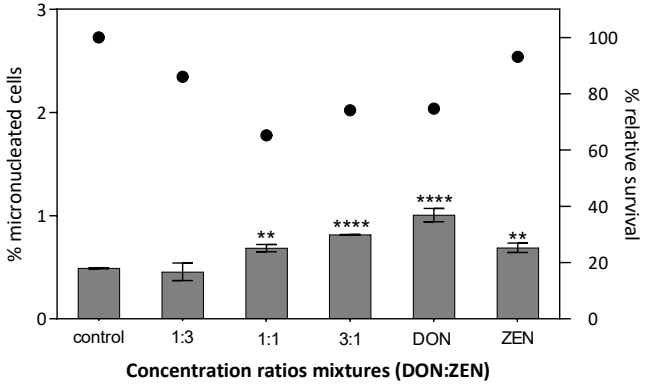
All mixtures are expected to induce the same effect if concentration addition applies

Results obtained with mixtures

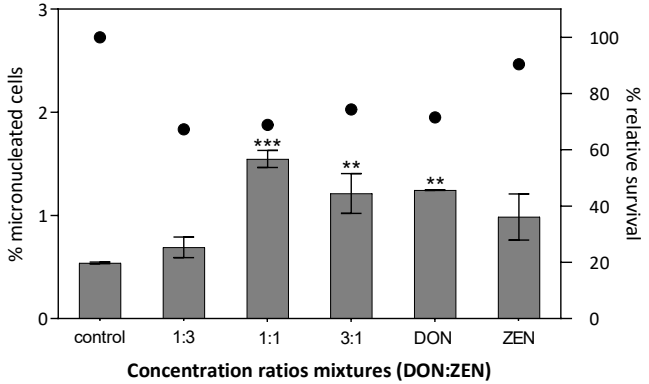
BMR 25%



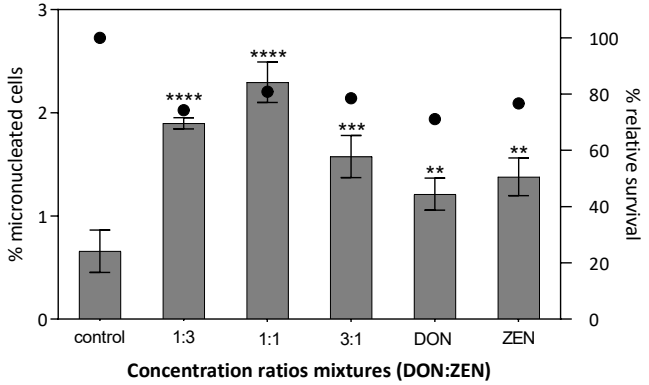
BMR 50%



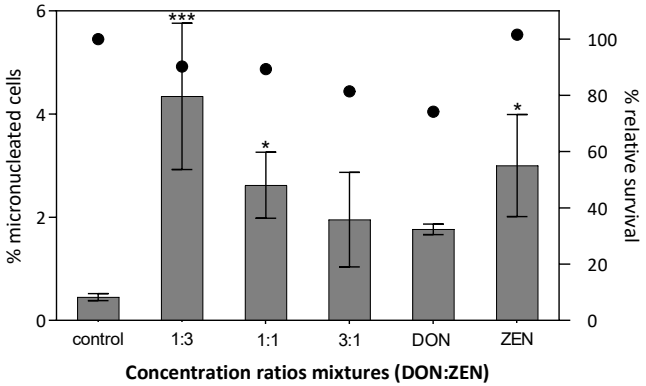
BMR 100%



BMR 150%

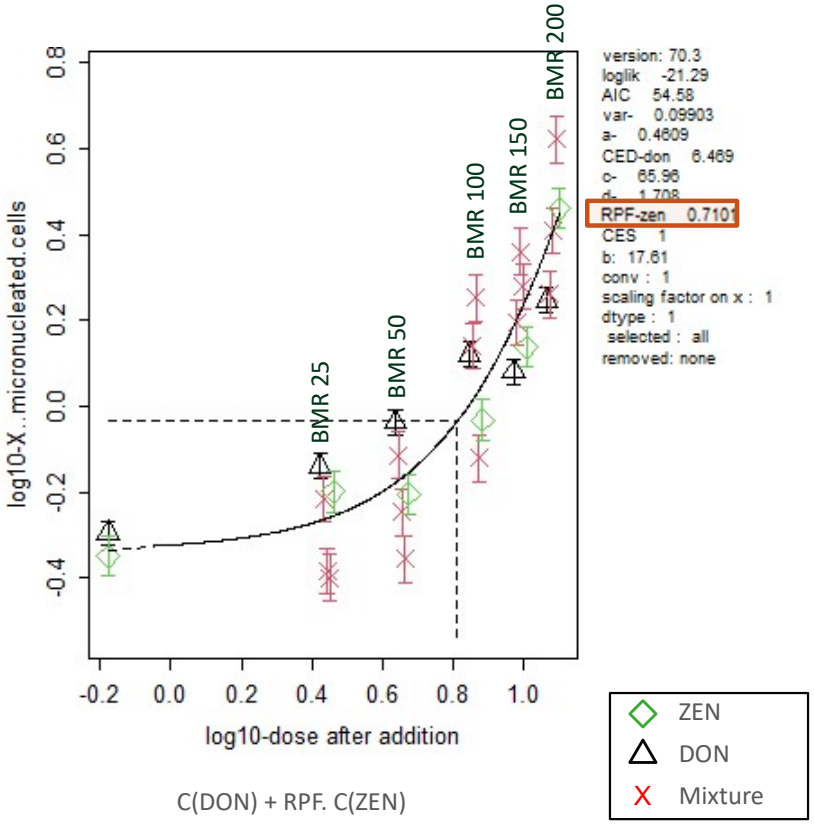


BMR 200%



Comparison of experimental and modelled results

PROAST package in R: dose-addition model 15



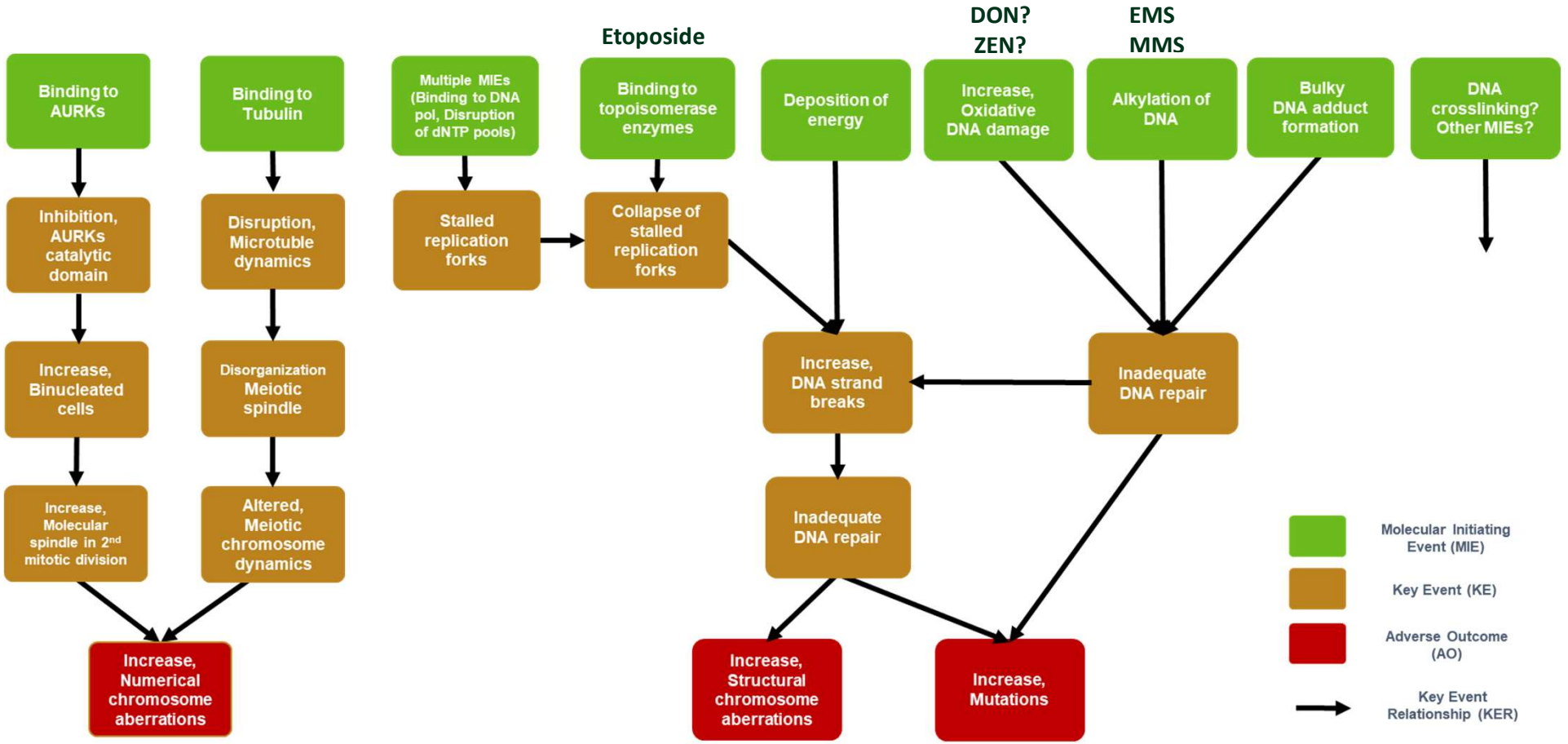
C(DON) + RPF. C(ZEN)

Results mixtures ✕ do not correlate consistently with /

- BMR 25%: close to background frequency
- BMR 200%: problems ZEN at high concentrations

Modes of action (MoA) underlying genotoxicity of DON and ZEN are not entirely clear!

Draft AOP network for genotoxicity



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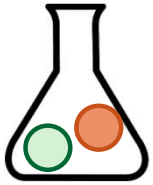
What's next?



Additional tests with mixtures of genotoxicants with different MoAs
(including mixtures containing > compounds)

Test with natural extracts to evaluate impact of 'unknown' mycotoxins
(and other compounds present in the extract)

Obtain more insights if principle of additivity applies for mixtures of genotoxicants with different MoA

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