DISCRISET

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People produce waste…

and need to dispose of it

Waste treatment

…according to waste toxicity

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HWD Waste Classification

H	criterion
H1	explosive
H2	oxidising
H3a	highly flammable
H3b	flammable
H4	Irritant
H5	harmful
H6	toxic
H7	carcinogenic
H8	corrosive
H9	Infectious
H10	Toxic for reproduction
H11	mutagenic
H12	release of (very) toxic gases
H13	Leachate with hazardous properties
H14	ecotoxic

1. Type of waste (e.g. hospital waste)
2. Hazardous properties of individual components when these are known (targeted analyses)
3. Complex wastes: if composition is unknown

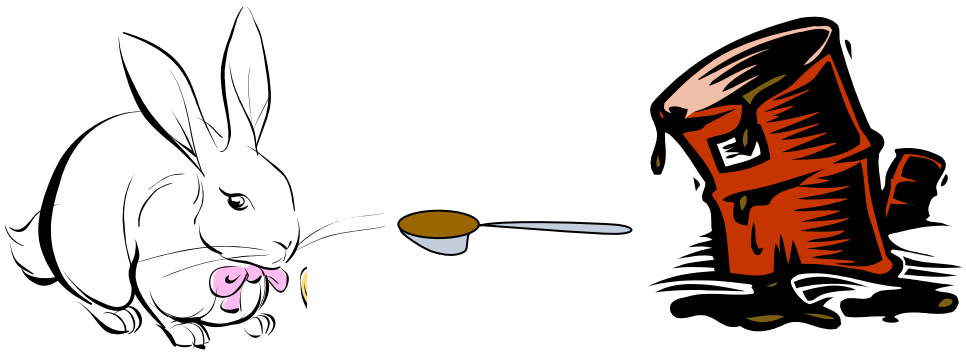
   ⇒ Test the hazardous properties of the waste

   ⇒ Annex V of DangerousSubstancesDirective 67/548/EEG

HWD Waste Classification

Annex V tests are often Mammalian tests!!

- Long test duration
- Expensive
- Unethical for waste assesment



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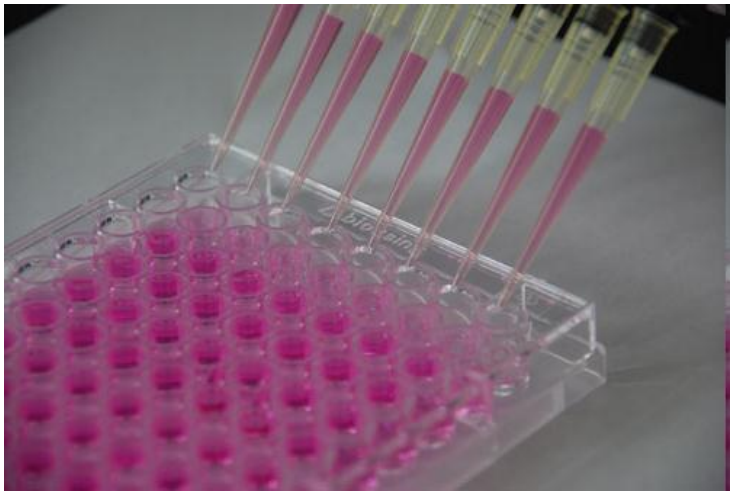
HWD Waste Classification

=> In vitro alternative methods

- **Ethical**
- **Fast**
- **Cheap**



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DISCRISET

Selecting a Set of (biological/chemical) fast screening tests that allow to discriminate between hazardous and non-hazardous waste!

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AIM

Selecting a Set of (biological/chemical) fast screening tests that allow to discriminate between hazardous and non-hazardous waste!

✓ according to HWD legislation

✓ as cheap as possible

✓as fast as possible

→ TESTING STRATEGY

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Suggested testing strategy

H	criterion
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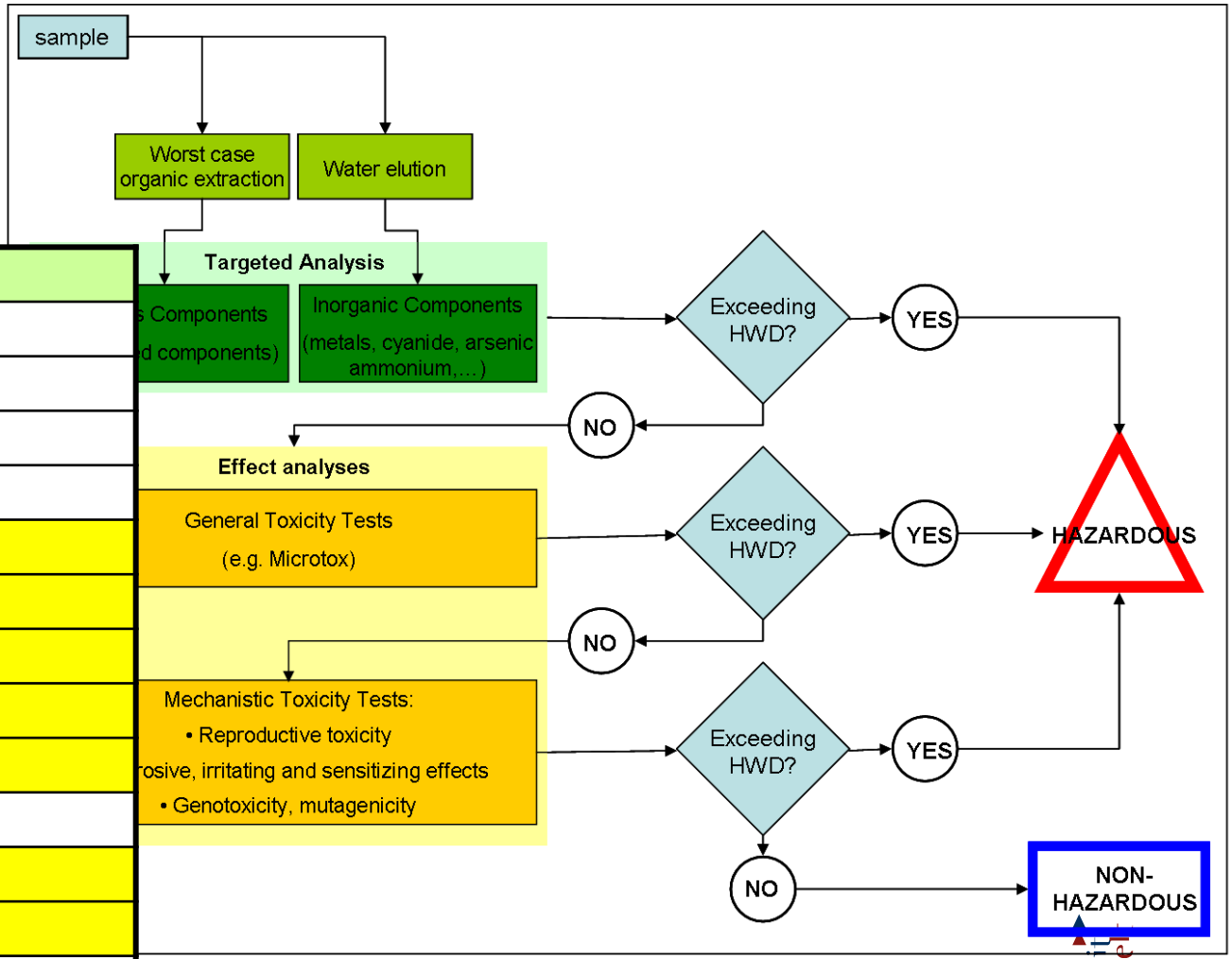

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Waste Samples

Sample	Source
fTEX	filter cake – input textile industry
fPAINT1	filter cake – input paint industry
fFOOD1	filter cake – input food industry
fFOOD2	filter cake – input food industry
fFOOD3	filter cake – input food industry
fPAINT2	filter cake – input paint industry
fPAINT3	filter cake – input paint industry
PSW	PCB containing shredder waste
SF	Shredder fluff
sDWTP	Sludge domestic WTP
FA	Fly ashes
WOOD	Wood
BA	Bottom ashes
sIWTP	Sludge industrial WTP
SA1	Soil additive
SA2	Soil additive

Pilot: filter residue samples

Wider range of samples

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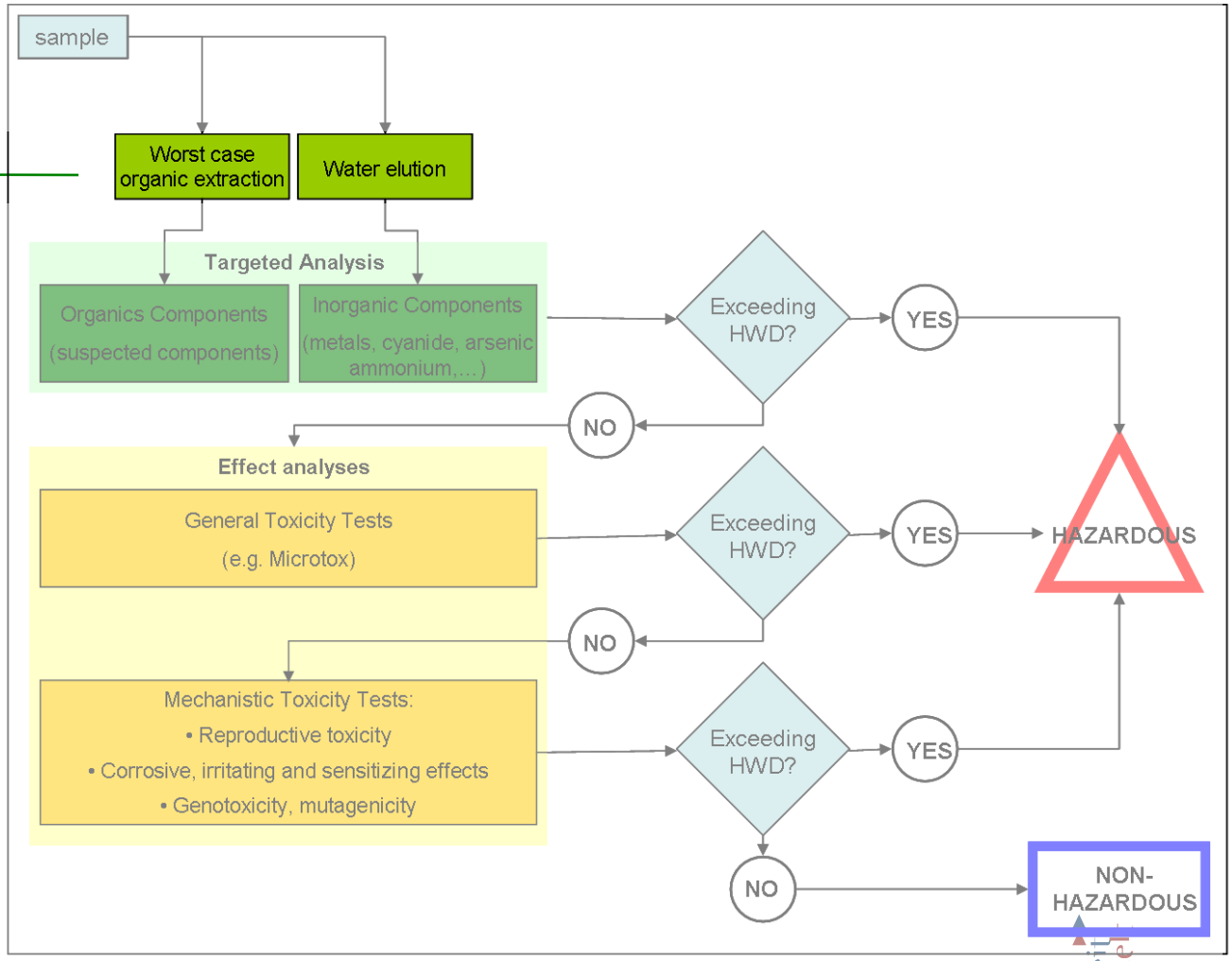
Suggested testing strategy

- **Leachate: inorganic analysis**
- **Aceton extraction + DMSO substitution**



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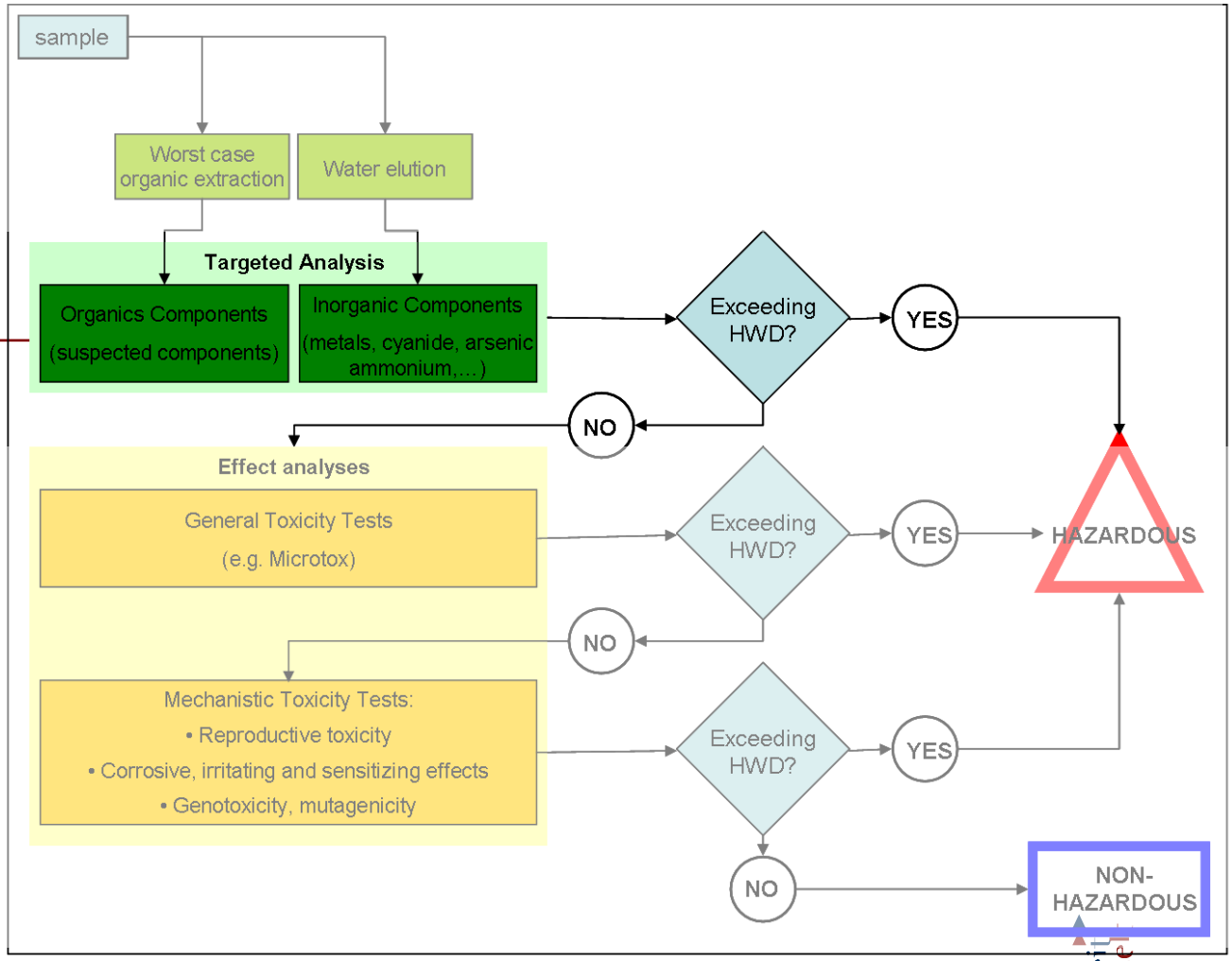
Suggested testing strategy

Aimed at targets with existing HWD limit



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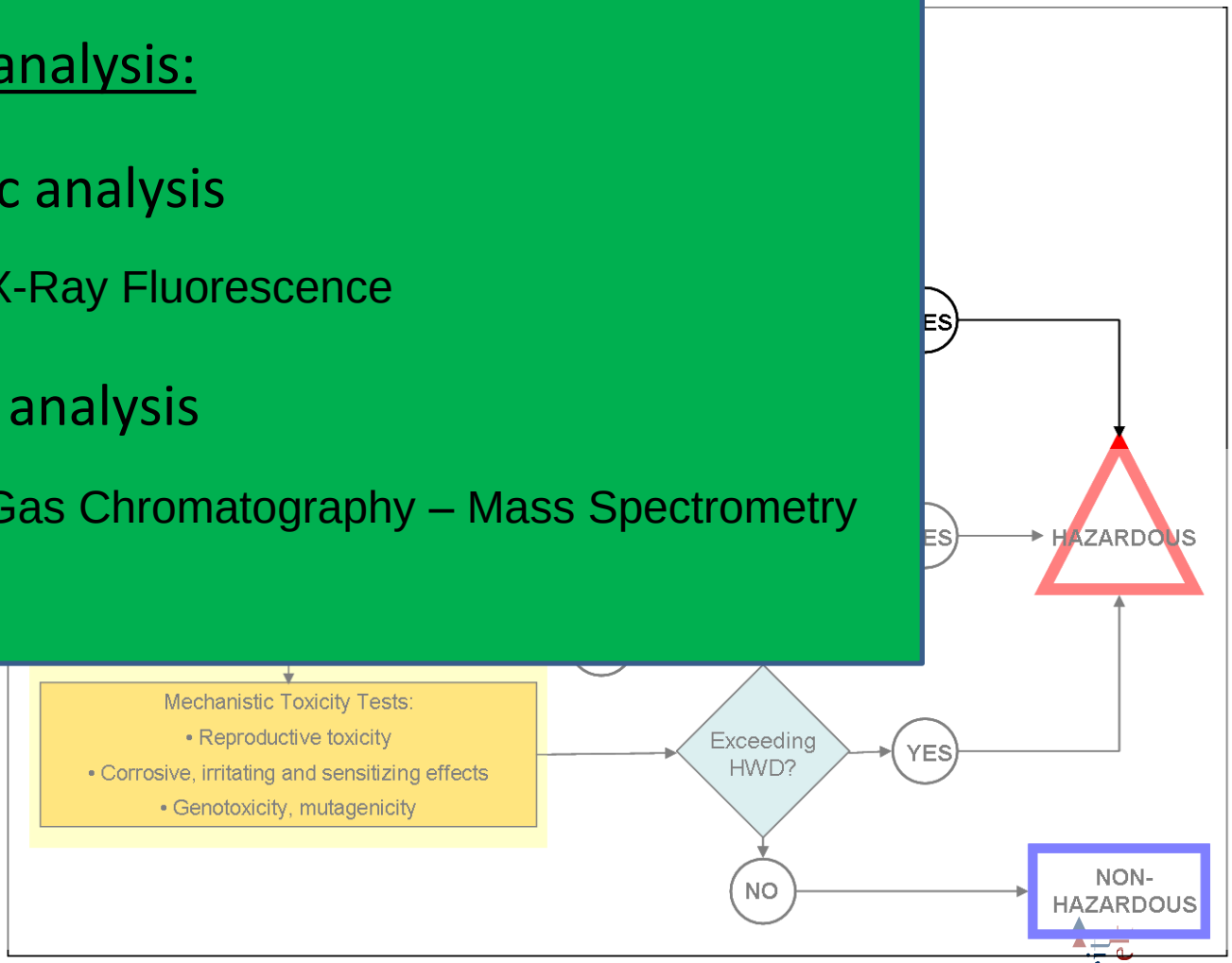
Suggested testing strategy

Targeted analysis:

- Inorganic analysis
  - ➡ X-Ray Fluorescence
- Organic analysis
  - ➡ Gas Chromatography – Mass Spectrometry



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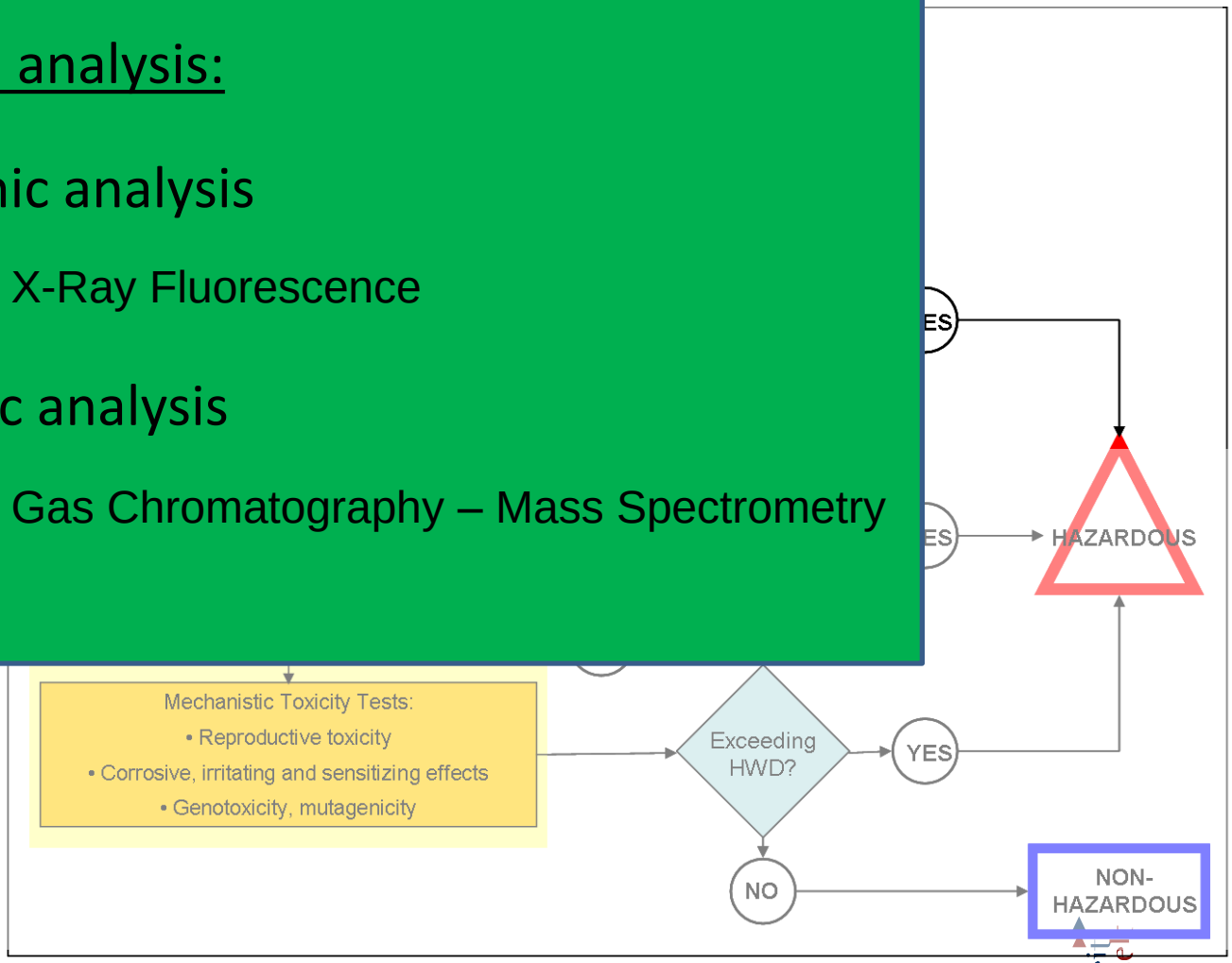

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Results XRF (inorganics)

		f	fP	fF	fF	fF	fP	fPAINT 2	PSW	SF	sDWTP	FA	WOOD	BA	sIWTP	SA1	SA2
								7.1	6.8	7.4	7.8	9.4	6	9.5	6.8	9.4	8.1
								3.44	2.49	0.84	1.85	4.83	<0.05	3.14	2.84	2.8	9.17
Si	%	1.86	2.55	0.3	1.38	0.53	3.49	0.76	6.82	4.98	20.5	19.6	0.09	21.4	6.9	12.11	7.208
P	%	0.051	0.85	1.43	1.24	2.2	0.93	0.67	0.23	0.239	0.17	0.39	100	0.497	4.93	0.017	0.049
S	µg/g				6520	8500	4840	7750	12220	27370	4460	7850	140	7190	8570	4483	4271
Cl	µg/g				708	1730	6370	9810	15190	10890	8140	6320	190	4330	880	91.8	19680
V	µg/g	<20	<20	<20	26.2	<20	55.3	<20	87	37	28	23.4	nm	82.4	49.5	54.3	533
Cr	µg/g	13.2	906	29.5	38.5	37.9	181	746	465	414	142	408	200	304	127	50.4	404
								8	86	68.8	<9.6	89.9	nm	154	47.7	<9	<23
								94	574	554	18.7	144	nm	423	50	24.4	42.6
								27	7700	1260	77.9	2130	2020	1630	609	19.1	15.8
Zn	µg/g	2150	5600	559	233	259	6410	4120	20820	15060	267	3190	36	2900	1960	46.3	26.2
Br	µg/g	11.8	13960	248	91.2	38.5	4200	67480	391	154	3	2.8	nm	16.3	49.7	1.4	171
Rb	µg/g	6.2			5.4	12.4	3.9	56.6	464	24.1	17.6	33.1	32.5	nm	26.3	32.8	nm	nm
Sr	µg/g	86.8			69.7	46.5	117	45	35.9	353	345	116	269	nm	413	345	70	96.5
Zr	µg/g	55.1			407	1140	667	830	664	521	172	148	174	nm	175	147	nm	nm
Nb	µg/g	1.7	1690		0.4	2.6	1.6	86.1	38.7	11.4	5.6	4.3	8.1	nm	9.1	6.13	nm	nm
								2.2	49.6	37.7	2.9	21.7	nm	13.3	21.6	nm	nm
								.5	76.1	48.8	6.3	6.7	nm	4.5	8.84	<3	<3
								56	547	298	10.3	274	nm	169	58.3	<9	30.3
								670	308	40.3	4.7	56.6	nm	65.3	21.6	<5	<5
Ba	µg/g	<18	481	62.9	54.7	<18	345	133	4150	961	234	1160	nm	1510	670		
Tl	µg/g	<2	19	<2	<2	<2	7.2	37.8	5	< 4.3	<2	<3	nm	<2.4	<2	<2	<2
Pb	µg/g	6.9	1150	13.6	7.8	5.8	129	203	3330	2770	85.3	1490	nm	958	257	49.1	48.7

Fast & relatively cheap

Comparison to existing HWD limits

HOWEVER

Speciation of elements is unknown

Combined effect is unknown

Very effective in evaluating known targets

Not conclusive for complex waste samples

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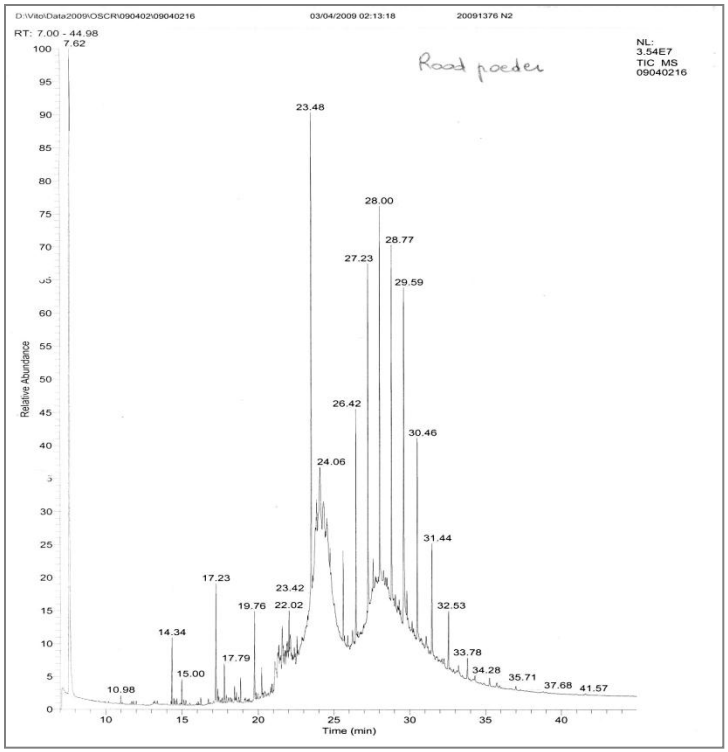
Results GC-MS (organics)



⇒**Many peaks (>200/sample!)**

⇒**Few identified (22%)**

⇒**Target needs to be known**

⇒ **Combined toxicity is not predictable**

⇒ **Quantitative analysis still needs to be performed**

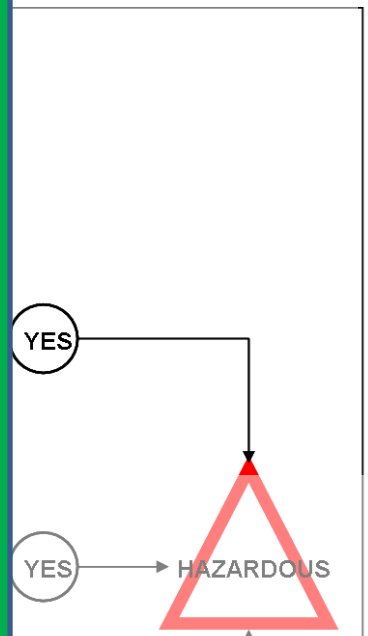
Can detect known (groups of) targets

Not conclusive for complex waste samples

Conclusion targeted analysis

Targeted analysis:

- Inorganic analysis
  - ➡ X-Ray Fluorescence
- Organic analysis
  - ➡ Gas Chromatography – Mass Spectrometry



- cheap and fast
- useful when targetting a specific (group of) compound(s)
- less applicable to complex samples

Conclusion targeted analysis

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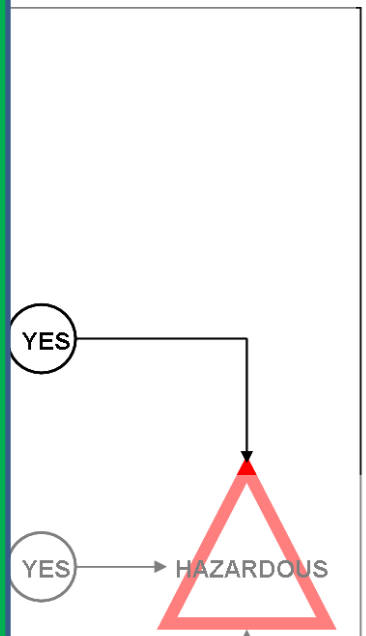

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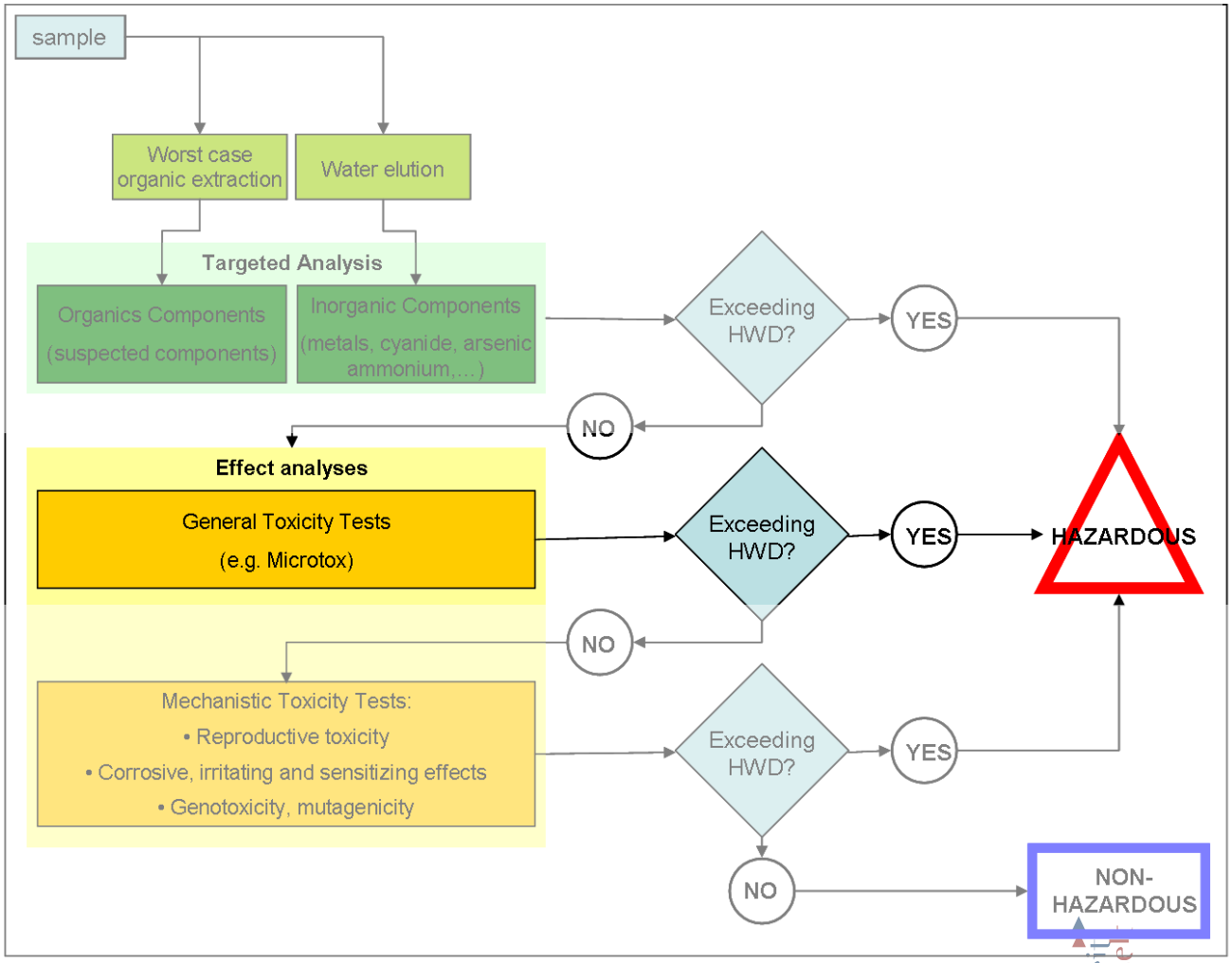
Suggested testing strategy



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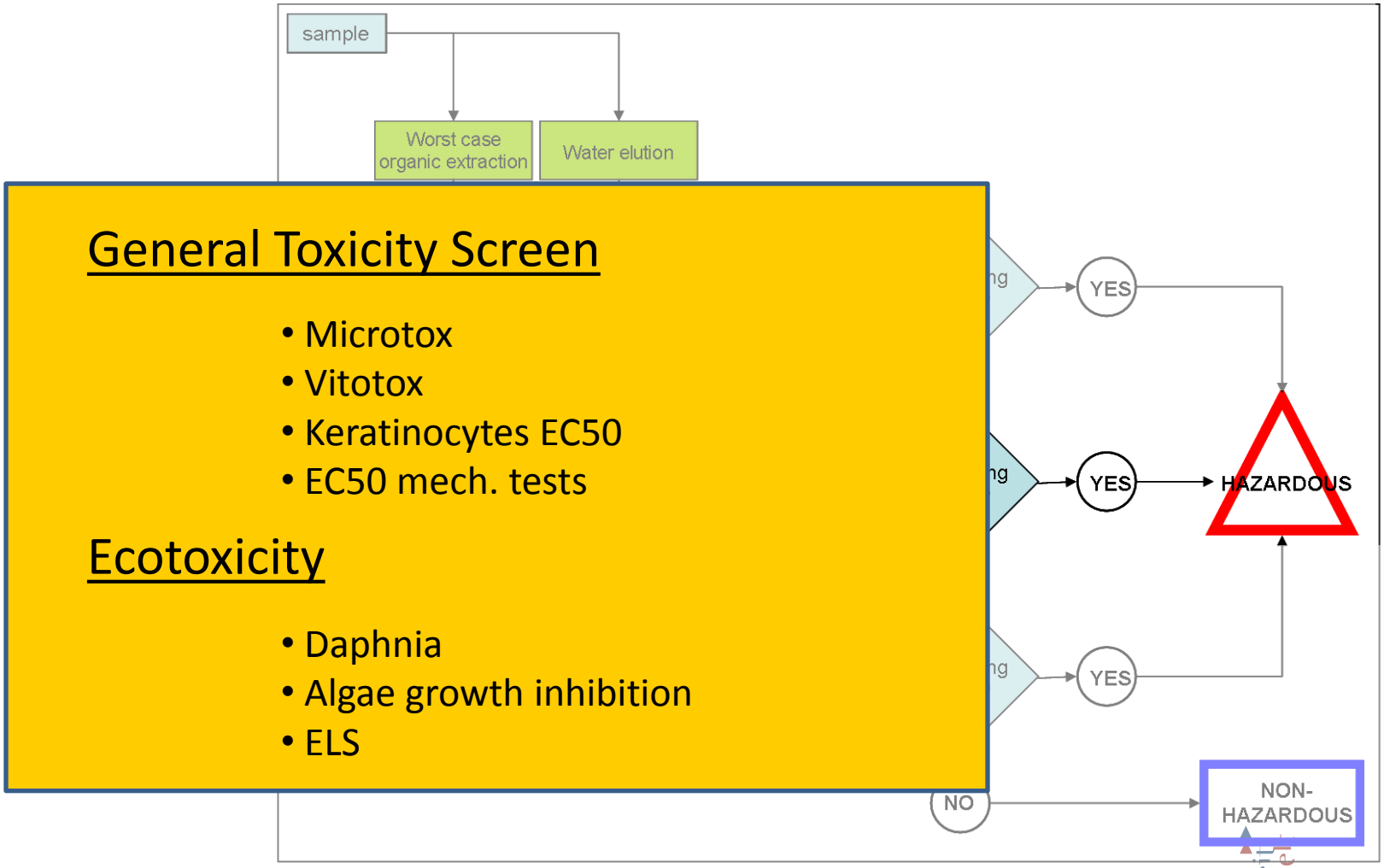

Bacterial tests:

- sensitive
- fast

Microtox: 30 min!

	Microtox	Vitotox	Daphnia	Algae (72h)	ELS	Cytotoxicity	Ames toxicity
	EC50 (30') (geq/l)	EC50(4h) (geq/l)	EC50 (48h) (geq/l)	% inhibition at 0.1%	LC50 (48h) (geq/L)	EC50 (72h) (geq/l)	EC50
	0.98	0.38	10.4	77.9	>	>	N
fPAINT1	0.19	0.1	0.05	97.5	0.19	0.2	CT
fFOOD1	16	-	>	0	>	>	N
fFOOD2	1.4	0.25	6.6	76.3	>	>	N
fFOOD3	2.02	1.65	8.2	61.3	>	>	N
fPAINT2	0.14	0.06	0.31	91	0.14	0. 46	CT
fPAINT3	0.08	0.06	0.21	85.3	0.18	0. 6	N
PSW	27	nm	>	0	>	>	N
SF	1.98	nm	2.14	92.6	6.1	>	N
sDWTP	5.12	nm	>	0	>	>	N
FA	82	nm	>	0	>	>	N
WOOD	0.6	nm	1.65	96	1.6	>	CT
BA	34	nm	>	0	>	>	N
sIWTP	1.1	nm	3.93	53	3.8	>	CT
SA1	>	nm	>	0	>	>	N
SA2	>	nm	>	0	>	>	N

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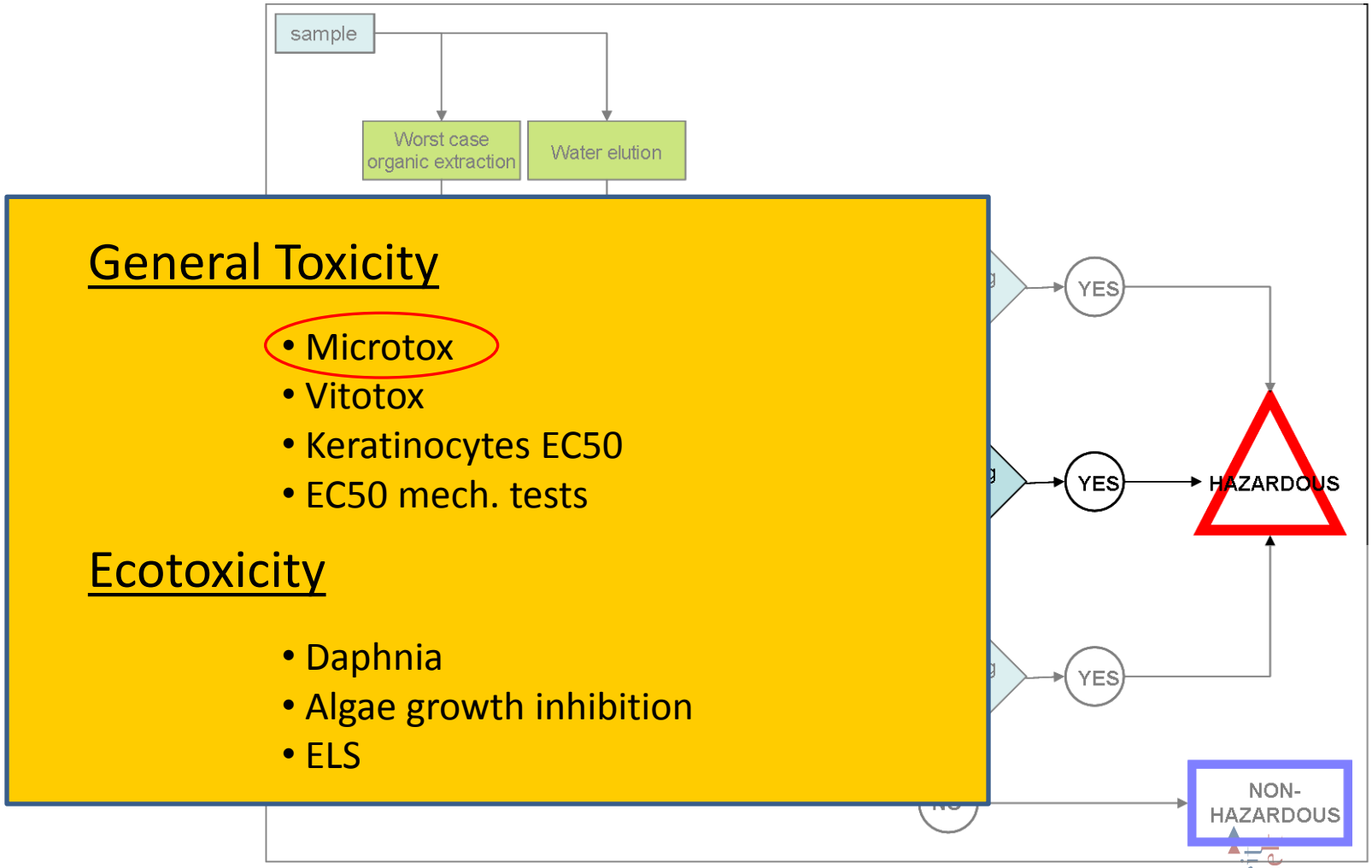
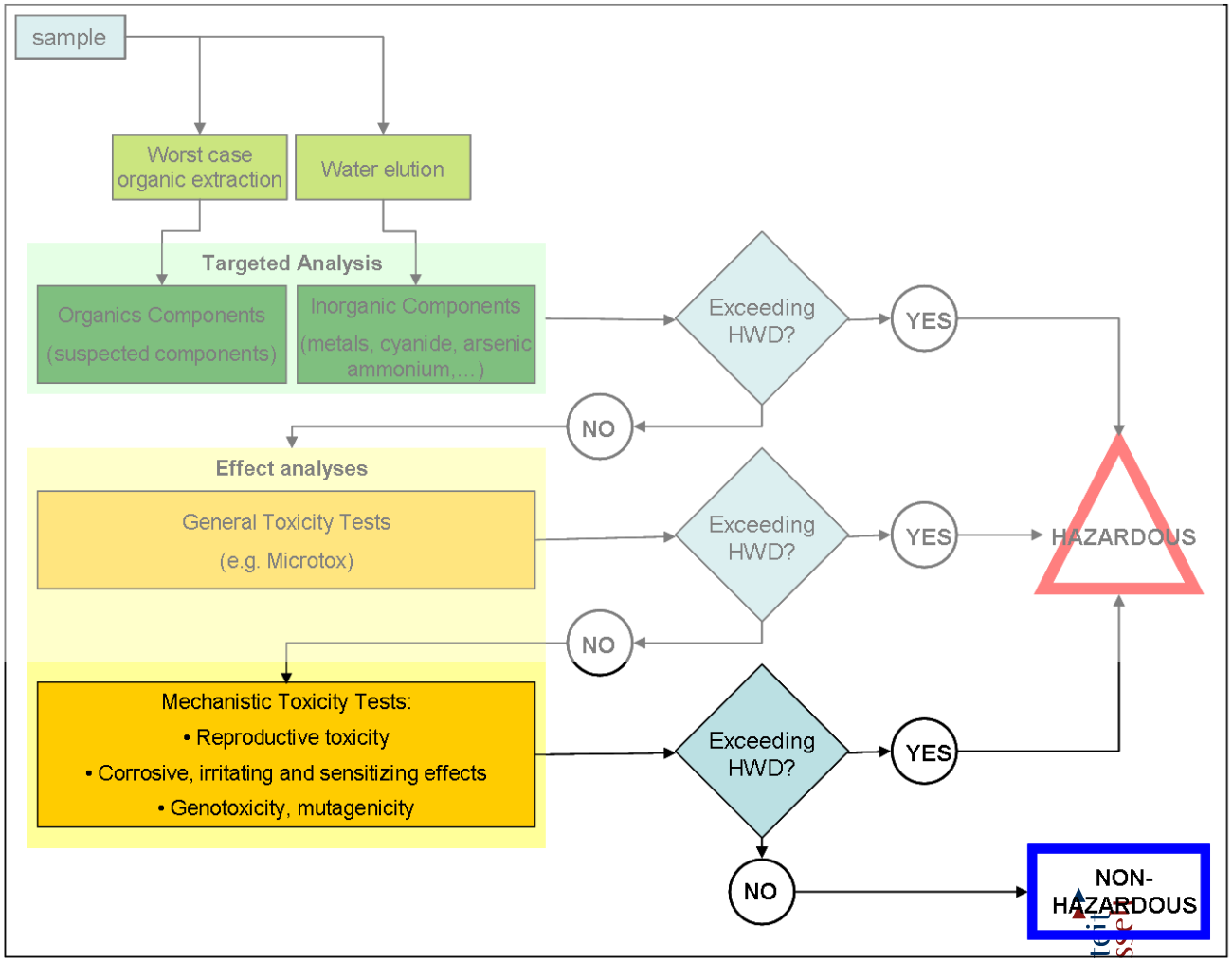
Suggested testing strategy



=> Suitable limits are being determined in benzene equivalents (Weltens et al., in preparation)

Suggested Testing Strategy



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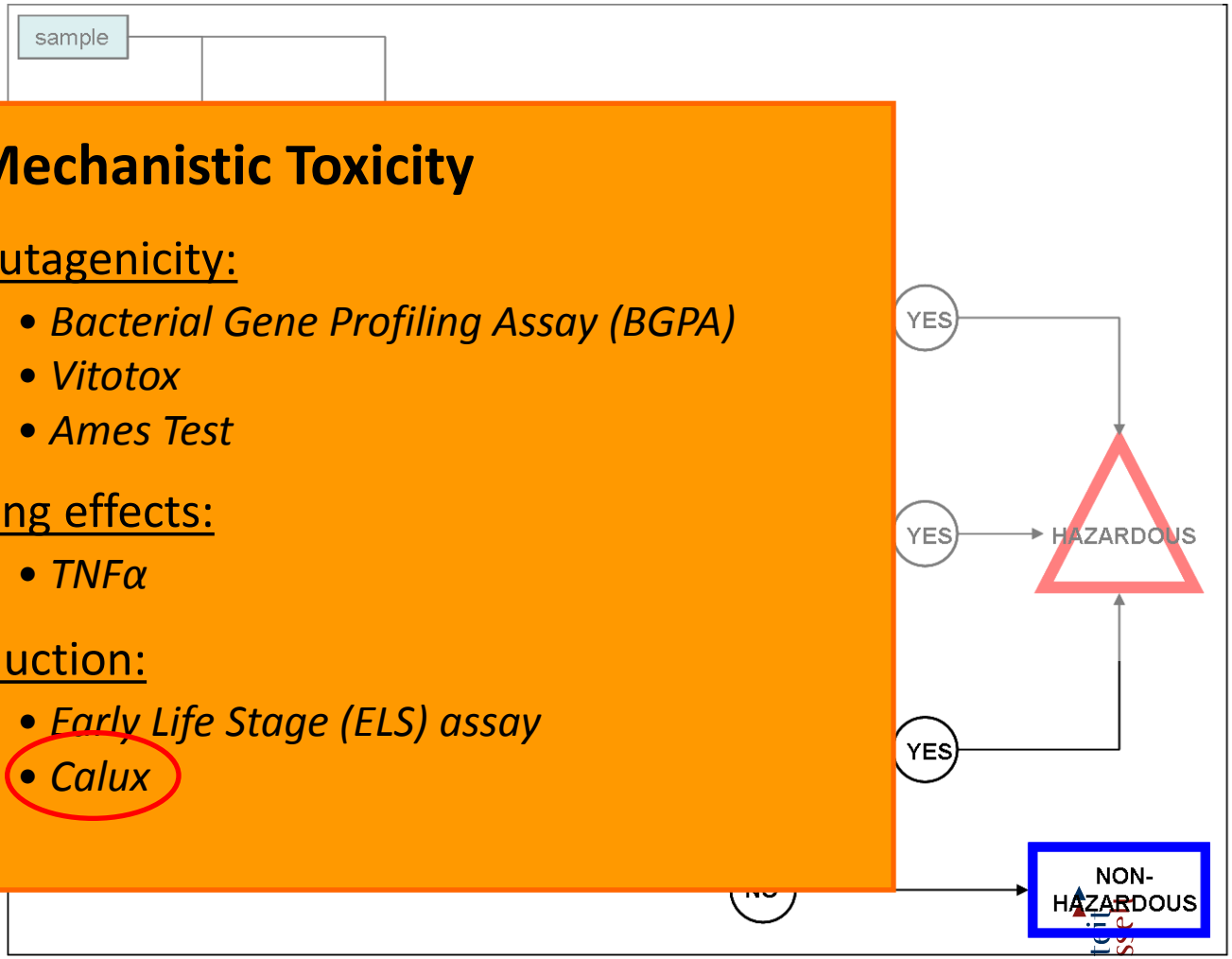

Mechanistic Toxicity

Genotoxicity / Mutagenicity:

- *Bacterial Gene Profiling Assay (BGPA)*
- *Vitotox*
- *Ames Test*

Irritant / sensitising effects:

- *TNFα*

Effects on reproduction:

- *Early Life Stage (ELS) assay*
- *Calux*

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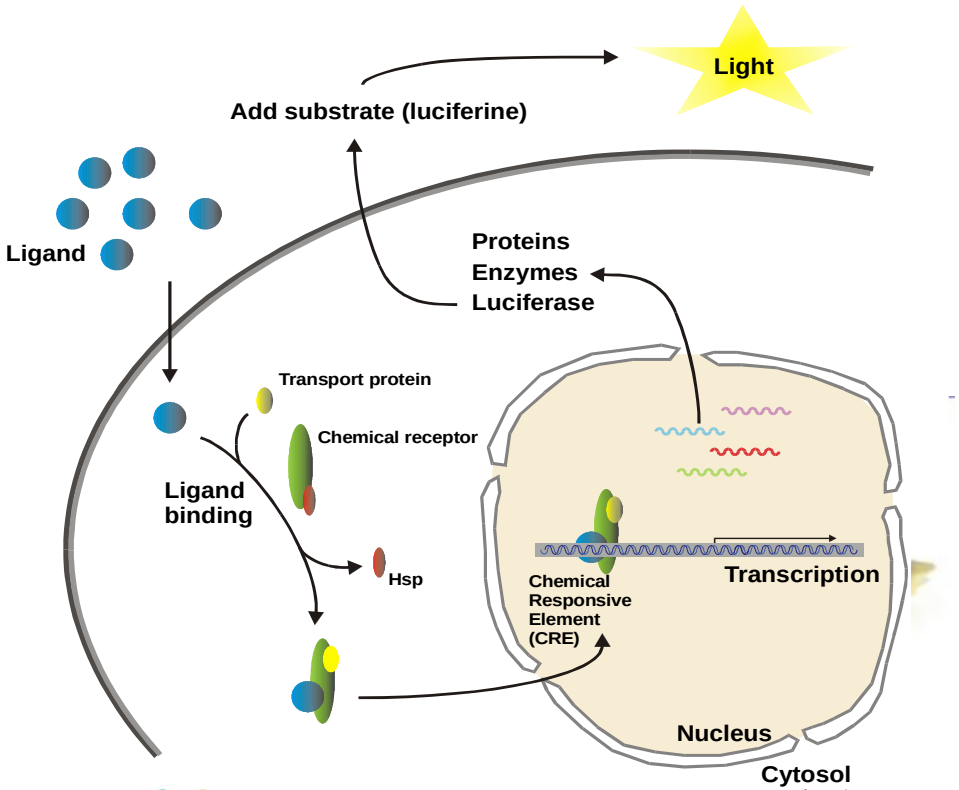
Mechanistic Toxicity - CALUX

Chemically Activated LUciferase eXpression

- ER
- ERα
- AR
- DR (AhR)



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Mechanistic Toxicity - CALUX

Chemically Activated LUciferase eXpression

- ER
- ERα
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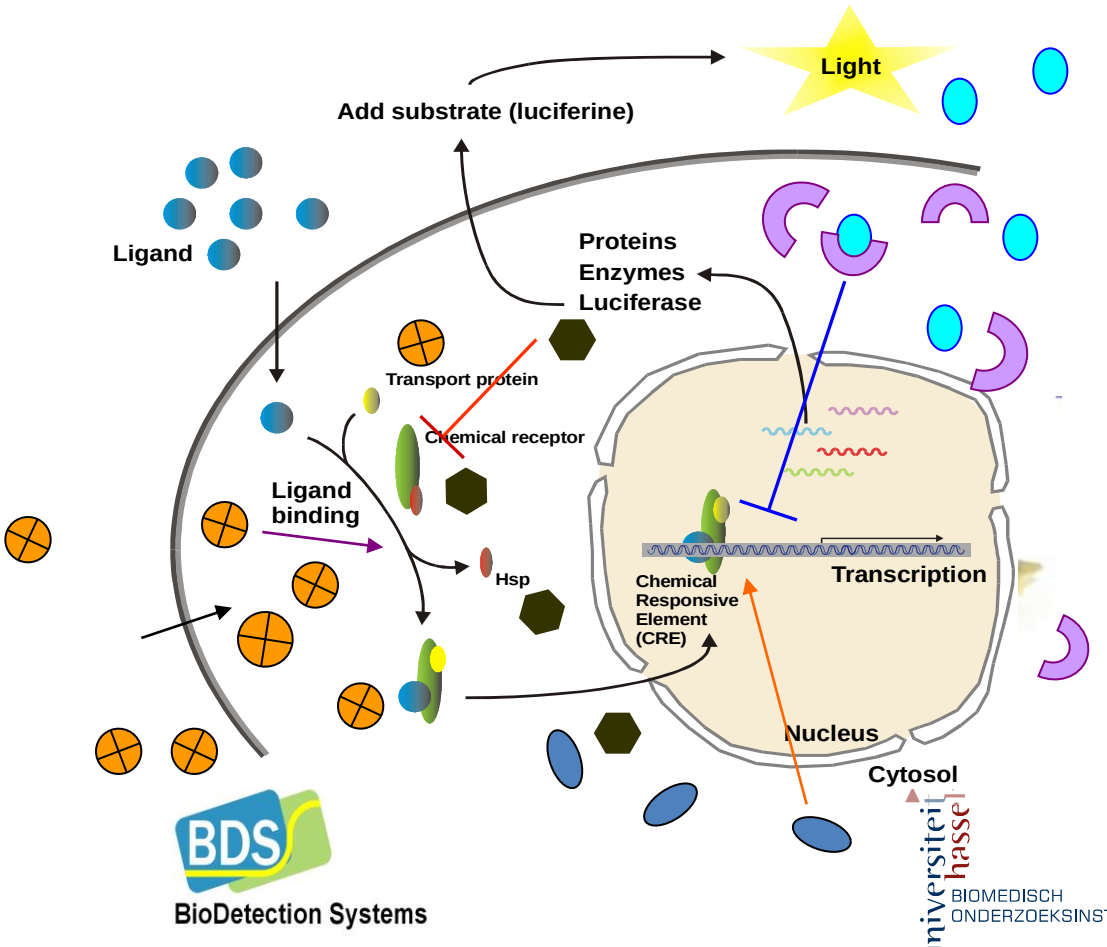
- DR (AhR)



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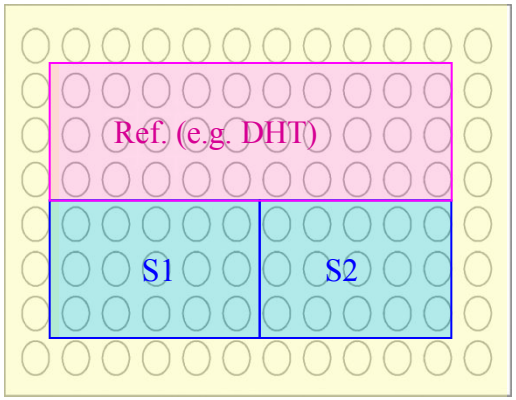
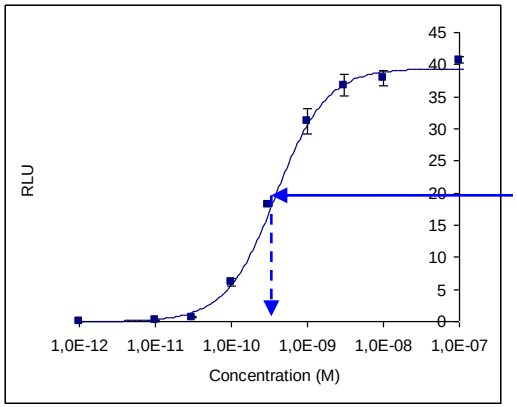
CALUX experimental set-up

- DMSO extracts of waste samples
- dilution series:
- 1 – 100 – 10.000 – 1.000.000 – 100.000.000
- This solution was added to cellmedium (e.g. 0,1 % for AR Calux)





Incubation Calux cells 24 – 48 h…

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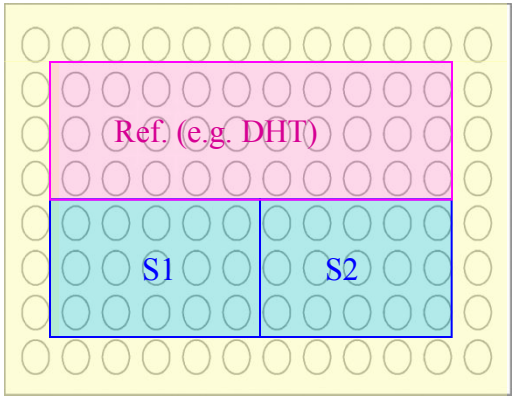
Indicative results

sample		DR ng TCDD eq/geq	ER ng EEQ /geq	ER α ng EEQ /geq	AR ng DHT eq/geq
fTEX	filter residue textile industry	1,07	0,04	0,12	< LOD
fPAINT1	filter residue paint industry	4,59	< 0,04	< 0,01	< LOD
fFOOD1	filter residue food industry	0,01	< 0,04	< 0,01	< LOD
fFOOD2	filter residue food industry	0,102	< 0,04	< 0,01	< LOD
fFOOD3	filter residue food industry	0,090	0,07	< 0,01	< LOD
fPAINT2	filter residue paint industry	7,64	16,44	4,21	< LOD
fPAINT3	filter residue paint industry	73,68	9,54	4,73	< LOD
PSW	PCB cont. shredder waste	51,33	< 0,04	4,70	< LOD
SF	Shredder fluff	64,41	< 0,04	1,20	< LOD
sDWTP	Sludge domestic WTP	< LOD	< 0,04	0,20	< LOD
FA	Fly ashes	< LOD	< 0,04	< 0,01	< LOD
WOOD	Wood	0,28	< 0,04	< 0,01	< LOD
BA	Bottom ashes	0,91	< 0,04	0,01	< LOD
sIWTP	Sludge industrial WTP	25,32	7,93	5,6	970
SA1	Ground additive	< LOD	< 0,04	< 0,01	< LOD
SA2	Ground additive	< LOD	< 0,04	< 0,01	< LOD

Indicative results

- DMSO extracts of waste samples
- dilution series:
- narrower range (eg. 1 – 3 – 10 – 30) to allow correct quantification
- This solution was added to cellmedium (e.g. 0,1 % for AR Calux)



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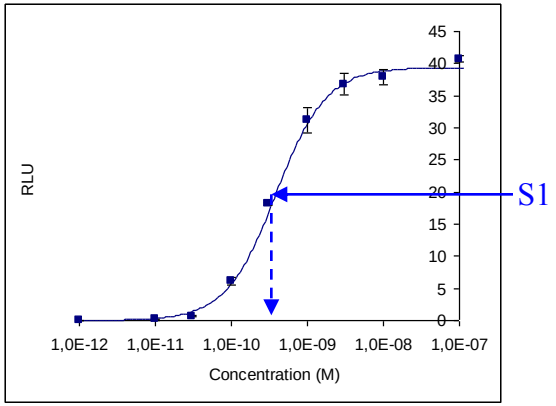

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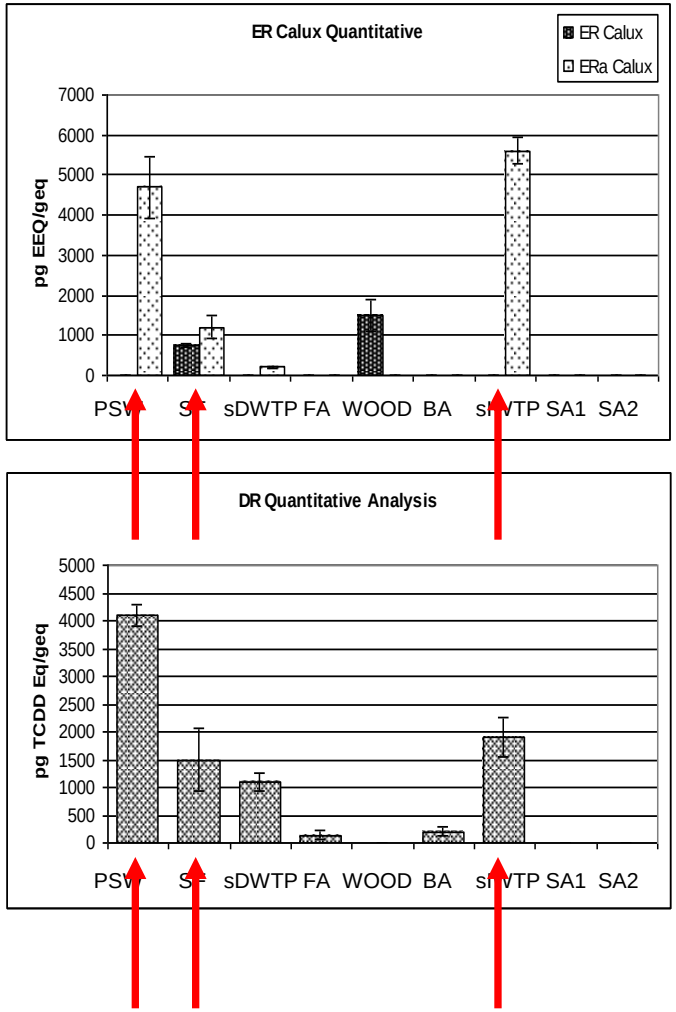
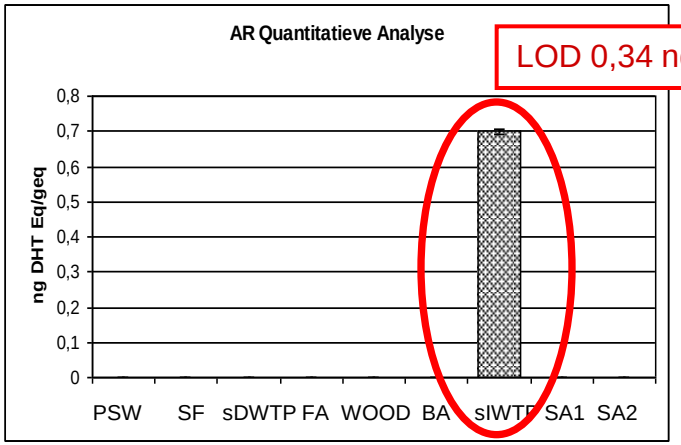
Quantitative Results Calux





LOD 0,34 ng DHTeq/geq

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Conclusion Calux

⇒Classification of waste based on endocrine activity is possible

⇒ LIMIT!!!

⇒ allow classification after only one assay

⇒ shorter time

⇒ diminished cost

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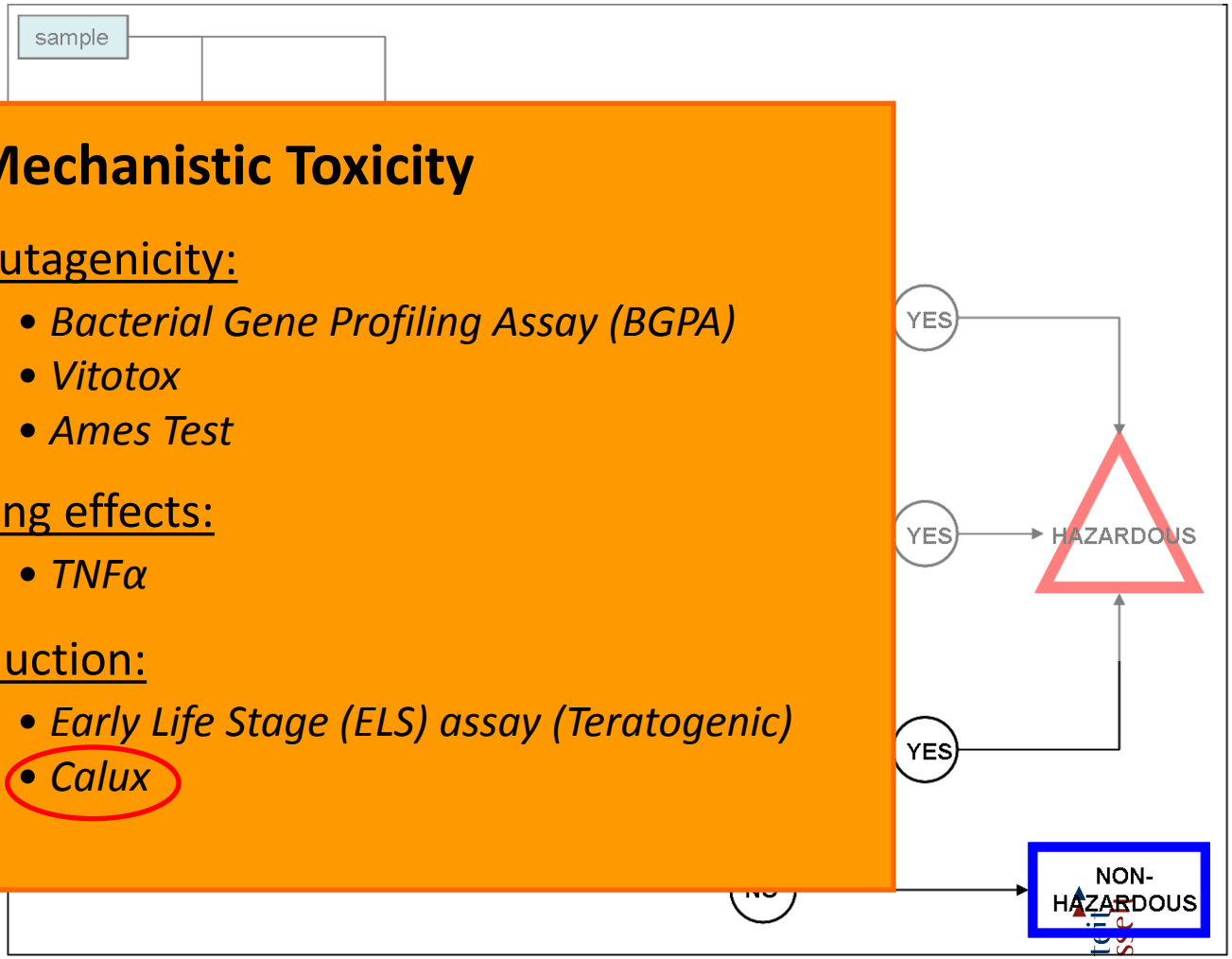
Suggested Testing Strategy



Mechanistic Toxicity

Genotoxicity / Mutagenicity:

- *Bacterial Gene Profiling Assay (BGPA)*
- *Vitotox*
- *Ames Test*

Irritant / sensitising effects:

- *TNFα*

Effects on reproduction:

- *Early Life Stage (ELS) assay (Teratogenic)*
- *Calux*

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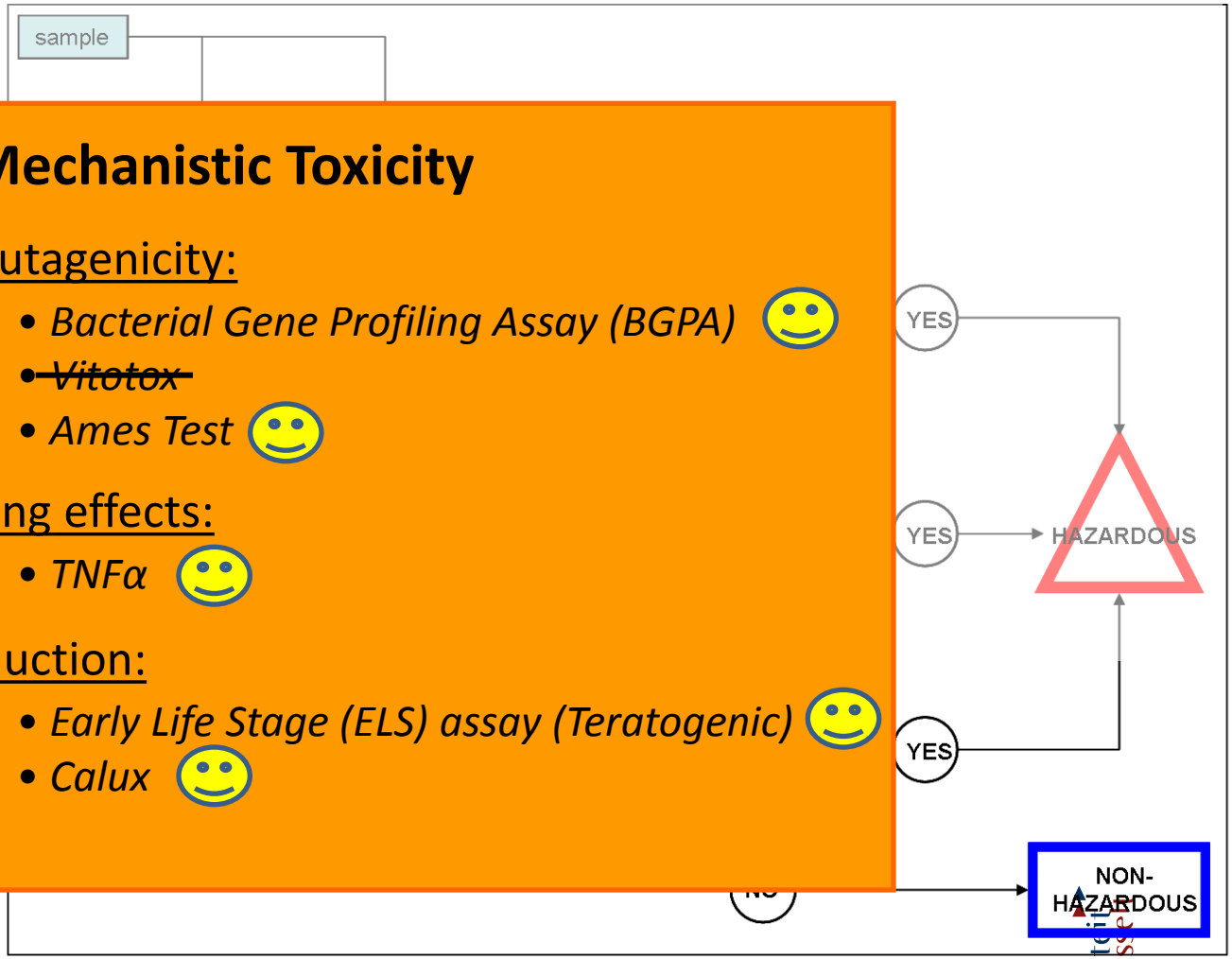
Mechanistic tests

- **Sensitive**
- **Fast**
- **Cheap**

Samples	Genotoxicity/ Mutagenicity				BGPA other endpoints			Irritating/ sensitizing	Effects on reproduction					
	BGPA DNA	Vitotox	Ames +S9	Ames –S9	Oxidative	Membrane	Cellular	TNFα	ELS	DR Calux	AR Calux	ER Calux	ERα Calux	YES
filter residue textile industry	■	■			■	■	■			■		■	■	
filter residue paint industry	■	■						■	■	■				
filter residue food industry										■				
filter residue food industry							■		■	■				
filter residue food industry						■	■			■		■		
filter residue paint industry		■	■	■	■			■	■	■		■	■	
filter residue paint industry	■	■	■		■	■		■	■	■		■	■	
PCB cont. shredder waste	■		■	■						■			■	
Shredder fluff	■		■	■					■	■		■	■	
Sludge domestic WTP	■				■								■	
Fly ashes					■									
Wood									■	■		■		
Bottom ashes	■			■	■					■				
Sludge industrial WTP	■			■	■	■			■	■	■		■	
Ground additive	■				■									
Ground additive														

Suggested Testing Strategy



Mechanistic Toxicity

Genotoxicity / Mutagenicity:

- *Bacterial Gene Profiling Assay (BGPA)*
- ~~*Vitotox*~~
- *Ames Test*

Irritant / sensitising effects:

- *TNFα*

Effects on reproduction:

- *Early Life Stage (ELS) assay (Teratogenic)*
- *Calux*

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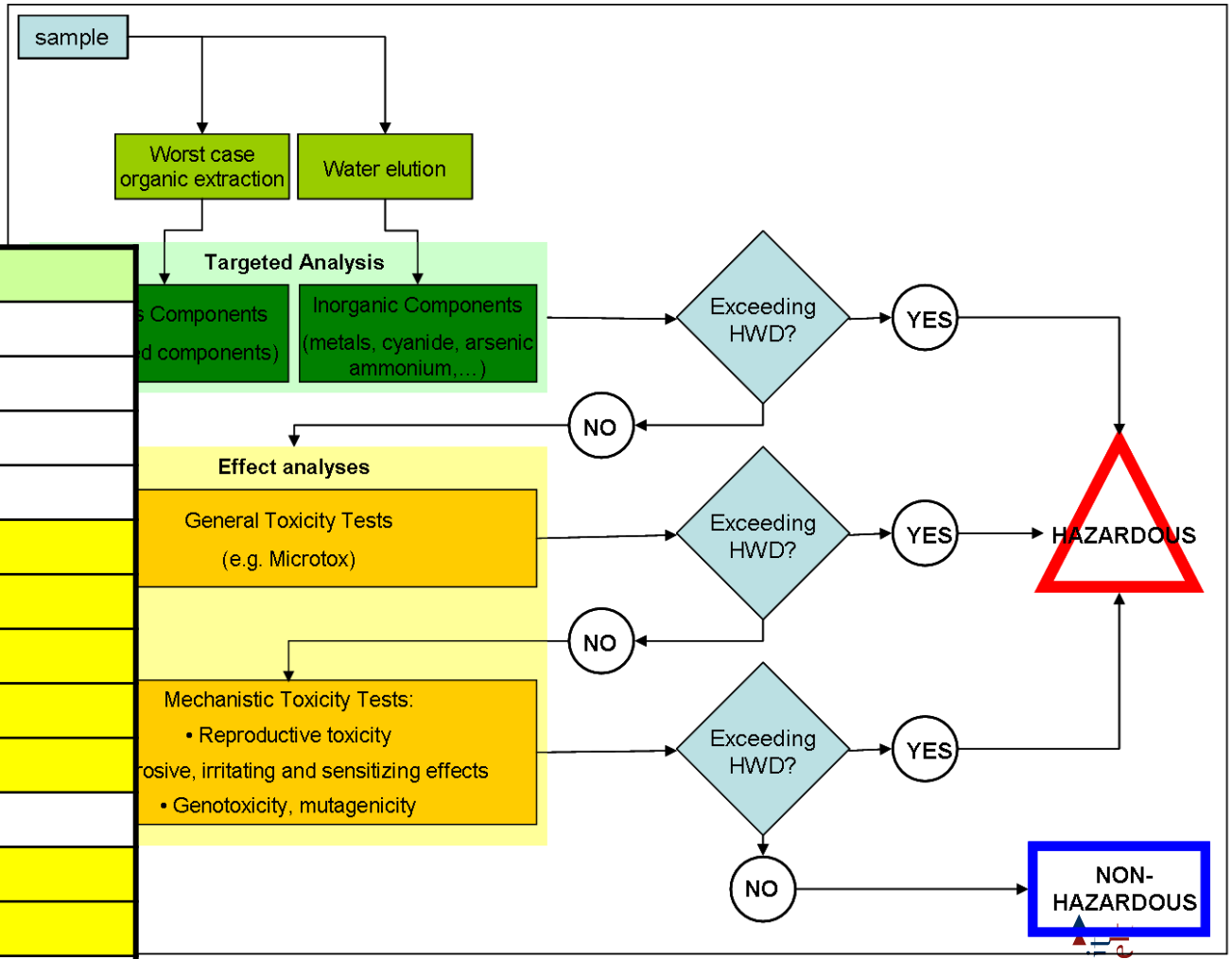
Suggested testing strategy

H	criterion
H1	explosive
H2	oxidising
H3a	highly flammable
H3b	flammable
H4	Irritant
H5	harmful
H6	toxic
H7	carcinogenic
H8	corrosive
H9	Infectious
H10	Toxic for reproduction
H11	mutagenic
H12	release of (very) toxic gases
H13	Leachate with hazardous properties
H14	ecotoxic



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Were all endpoints met?

H	criterion	
H1	explosive	
H2	oxidising	
H3a	highly flammable	
H3b	flammable	
H4	Irritant	=> TNFα
H5	harmful	=> Microtox
H6	toxic	
H7	carcinogenic	=> Ames, BGPA
H8	corrosive	
H9	Infectious	
H10	Toxic for reproduction	=> **Calux**, ELS
H11	mutagenic	=> Ames, BGPA
H12	release of (very) toxic gases	
H13	Leachate with hazardous properties	
H14	ecotoxic	=> Microtox

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Summary

- **Targeted analysis is very fast and cheap**
  - Useful in specific cases (waste possibly containing known compounds)
  - For complex waste not conclusive (speciation unknown, cumulative effects unknown)
- **General toxicity screening can be done quickly and efficiently with Microtox**
  - Classfication of very toxic samples in 30 min
  - No information on specific toxic effects
- **Mechanistic toxicity assays such as Calux allow classification of samples with specific toxic effects**
  - No need to identify compounds
  - Cumulative effects are clear

Remaining challenges:

- Missing endpoints (e.g. corrosive, embryonic implantation, long-term fertility effects…)
- Limits!

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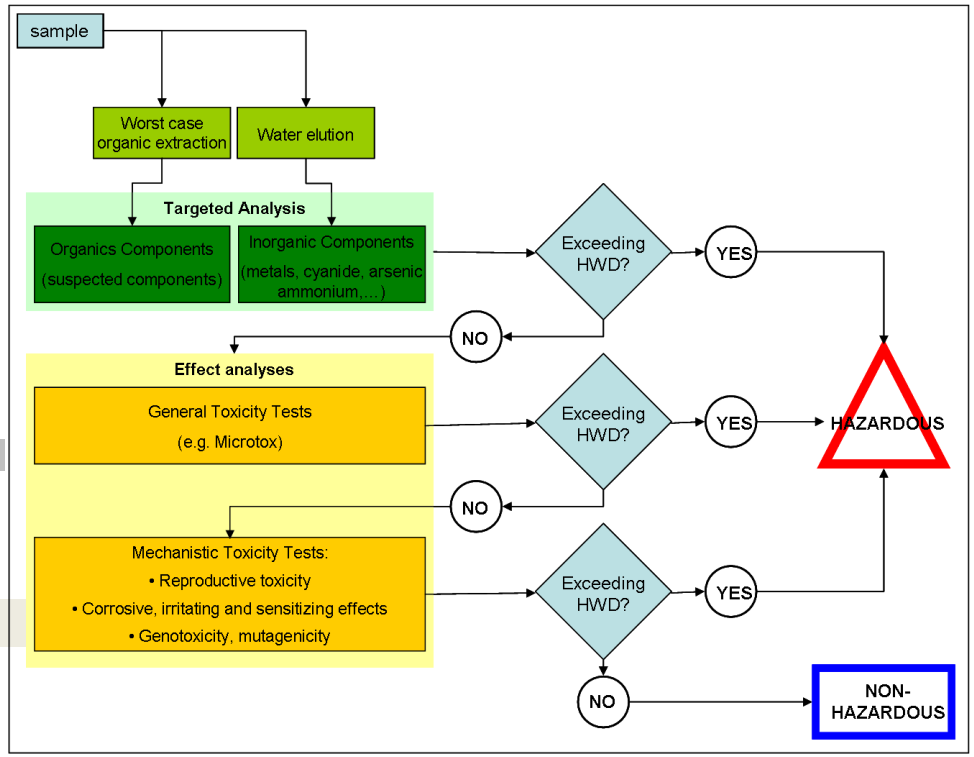
Conclusion

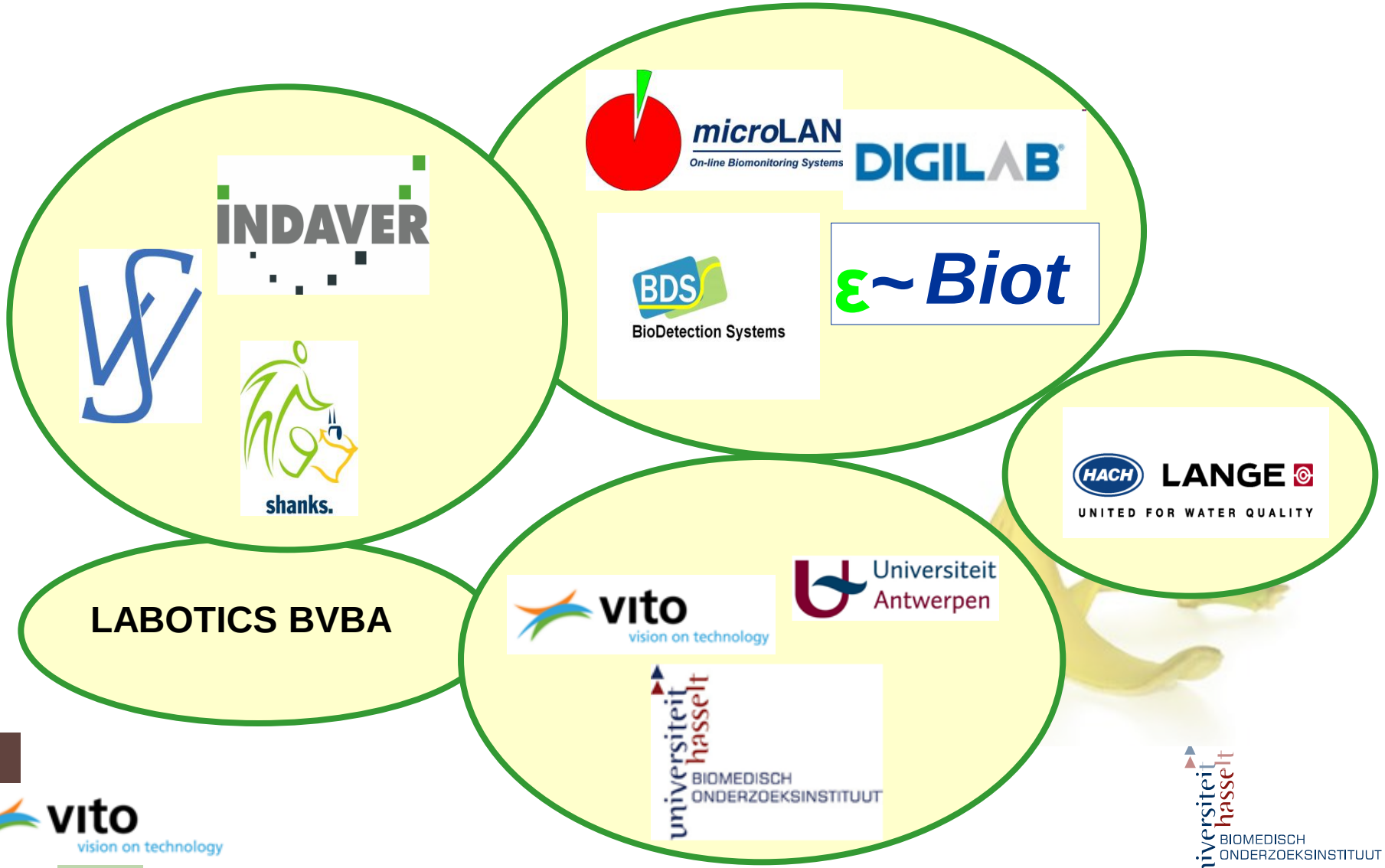


- Testing Strategy works!
- Samples probably contaminated with known toxicants can be tested by targeted analysis
- Very complex samples can be analysed using XRF + effect based analysis

⇒ Weltens et al. 2012 (Waste Management Journal)

⇒ Deprez et al. 2012 (Waste Management Journal)

DISCRISET



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Thank you!

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