

Industry board meeting, project “MIGRATOX”; September 17th, 2023, Vienna (Hybrid Event)

10:30	Welcome and introduction	13:35	C. Kirchnawy & L. Prielinger <i>“In-vitro screening results of the miniaturized Ames test on printed FCMs”</i>
10:45	Christian Kirchnawy (OFI) <i>“Migratox Project: Recap”</i>	14:00	Open Discussion
11:00	Sander Koster (Nestlé Research Centre) <i>“Improving the identification of unknowns in NIAS screenings”</i>	14:15	Elisa Mayrhofer (OFI) <i>“Application of the Migratox method for mineral oil risk assessment”</i>
11:25	Th. Czerny & L. Prielinger (FH Campus Vienna) <i>“Ames Sense: Analysis of plastic extracts with an improved miniaturized Ames test”</i>	14:35	Silvia Apprich & C. Kirchnawy <i>“Summary & Outlook on final steps”</i>
11:50	Open Discussion	14:50	Open Discussion, Opportunity to bring in your own ideas/wishes
12:10	Snacks & Networking	15:00	Coffee & Networking
13:10	Evert Delbanco (Siegwerk) <i>“Safety evaluation of contact sensitive printing inks and future regulatory challenges”</i>		

“OFI and companies participating at OFI meetings shall not enter into any discussion, activity or conduct that may infringe, on its part or on the part of its members, any applicable competition law.

By way of example, members shall not discuss, communicate or exchange, any commercially sensitive information, including information relating to prices, marketing and advertising strategy, costs and revenues, trading terms and conditions with third parties, including purchasing strategy, terms of supply, trade programs, or distribution strategy. Please take note that taking part in today’s meeting is subject to having read and understood the OFI Business Conduct Guideline for events and meetings with competition law and antitrust relevant contents.”

Why did we start the Migratox Project?

Substance	Retention time [min]	Qual %	Concentration [µg/kg] **	CAS Nr.	Listed in VO (EU) Nr. 10/2011.	SML / SML(T) [mg/kg]	Cramer Class	Structural Alert Mutagenicity
o-Cymene	15,00	97	28*	527-84-4	-	-	I	No
Unidentified hydrocarbon	15,78	<80	70	-	-	-	-	-
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	16,78	95	28	586-62-9	-	-	I	No
Unidentified hydrocarbon	16,97	<80	14	-	-	-	-	-
Dodecane	19,48	96	155*	112-40-3	-	-	I	No
Decanal	19,65	99	21	112-31-2	-	-	I	Yes
Unidentified hydrocarbon	21,48	<80	28	-	-	-	-	-
Isobornyl acetate	21,94	99	49*	125-12-2	-	-	I	No
Unknown substance	22,04	<80	31*	-	-	-	-	-
4-tert-Butylcyclohexyl acetate	23,76	91	34*	32210-23-4				
1-Tetradecene	24,02	96	22	1120-36-1				
Tetradecane	24,20	97	35*	629-59-4				
Dodecanal	24,44	91	77	112-54-9				
Diphenyl ether	24,62	97	17	101-84-8				
Unknown substance	25,04	<80	13	-				
Caryophyllene	25,21	99	68*	87-44-5				
Trans-.alpha.-Bergamotene	25,31	99	73	13474-59-4				

Rt [min]	Sum Formula	Mass (m/z)	Concentration [µg/kg]
11.2	C9H18O2	157.1240	49
12.0	C14H22O	205.1602	5
13.7	C14H22O	205.1604	18
17.2	C14H28O2	227.2021	73
18.8	C16H32O2	255.2335	60
19.0	C18H34O2	281.2487	9.6
20.1	C18H36O2	283.2645	92
23.7	C28H43O4P	473.2829	41

Why did we start the Migratox Project?

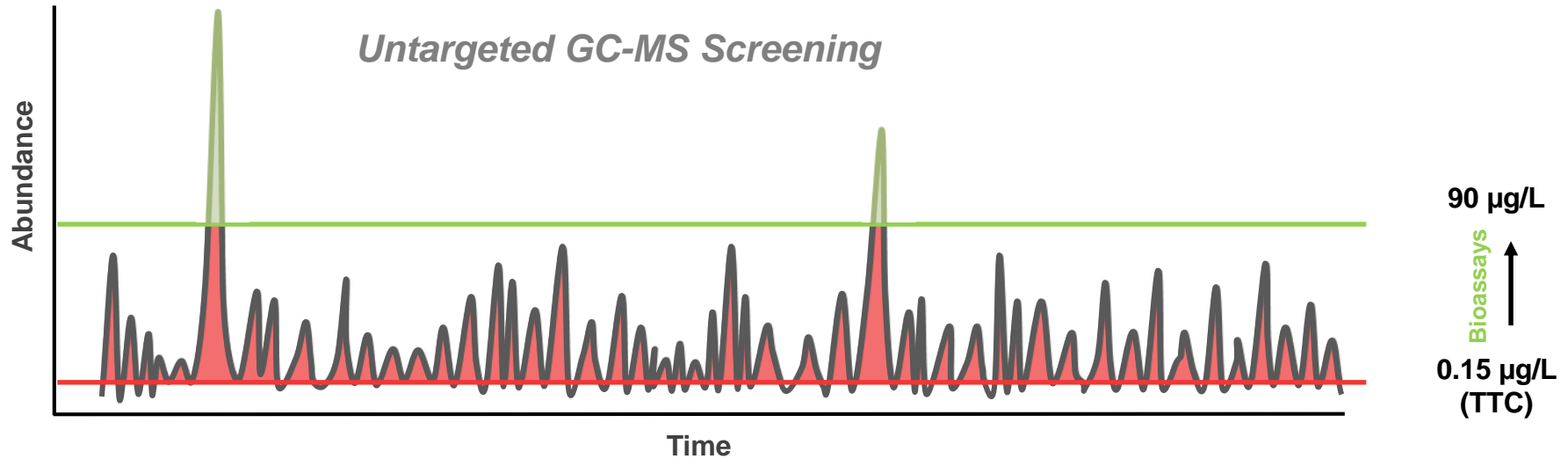
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Main Goal of the Migratox Project:

Develop an additional tool to help with NIAS safety assessment!

Dodecane	24,62	97	17	101-84-8	13.7	C14H22O	205.1604	18
Decane	25,04	<80	13	-	17.2	C14H28O2	227.2021	73
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1-Tetradecanol								
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Dodecane								

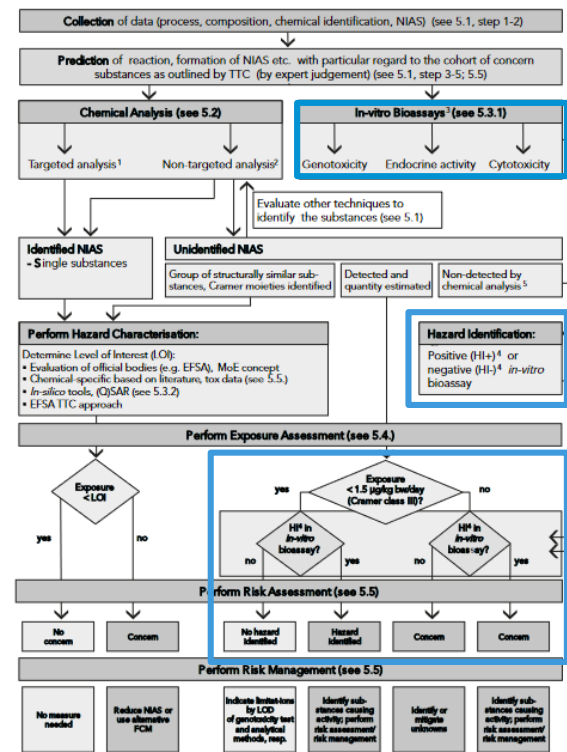
Risk Assessment of unidentified NIAS



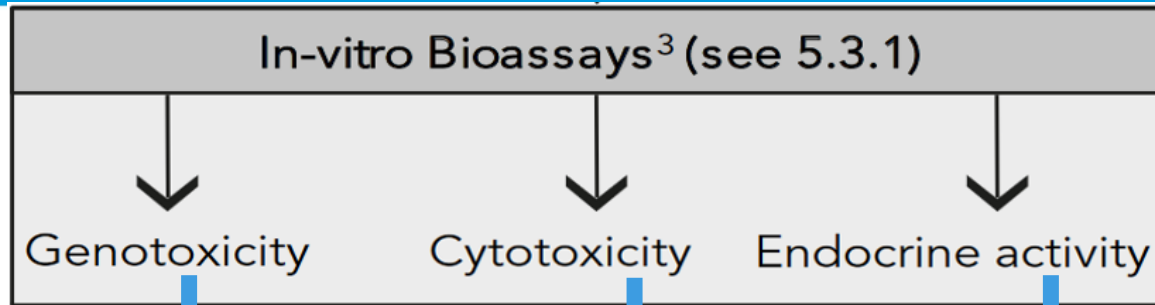
ILSI Europe Report Series

GUIDANCE ON BEST PRACTICES ON THE RISK ASSESSMENT OF NON INTENTIONALLY ADDED SUBSTANCES (NIAS) IN FOOD CONTACT MATERIALS AND ARTICLES

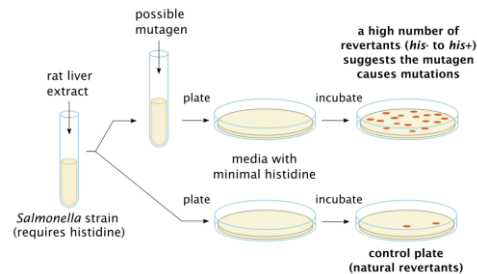
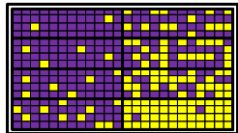
Figure 1: Flowchart for the risk assessment of NIAS (may also apply to substances other than NIAS).



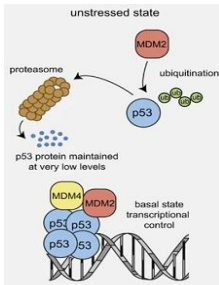
ILSI – NIAS report suggests bioassays for unidentified NIAS



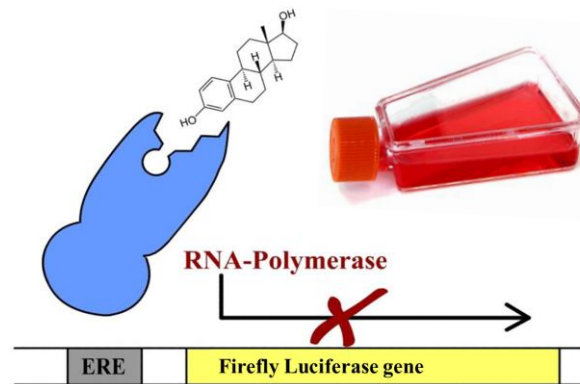
Ames-Test: Bacteria Test DNA-reactive Genotoxicity



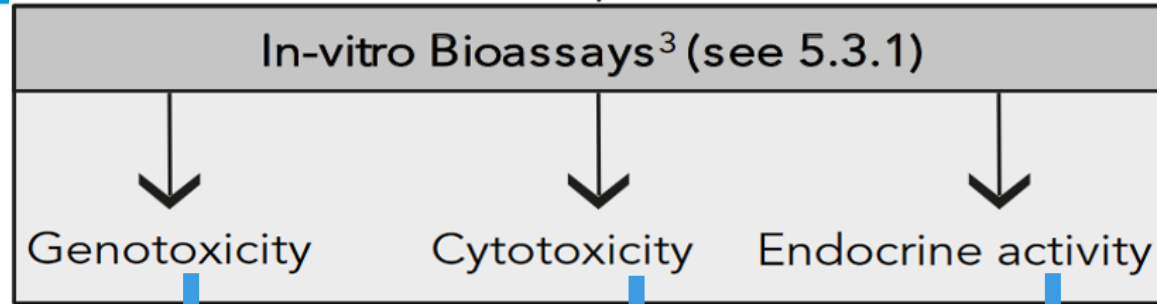
HepG2 Assay, P53 CALUX, Micronucleus, High Content Screening: Cell Culture Tests for Chromosomal Damage



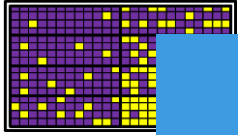
ER-CALUX, (Anti)AR-CALUX: Cell culture Test, includes Cytotoxicity



ILSI – NIAS report suggests bioassays for unidentified NIAS



Ames-Test: Bacteria Test
DNA-reactive



**HepG2 Assay, P53
CALUX, Micronucleus,**

ER-CALUX, (Anti)AR-CALUX:
Cell culture Test, includes Cytotoxicity

Goal for selection of Bioassay Battery:
„As many bioassays as necessary but
as few as possible“

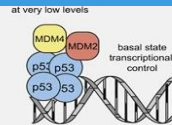
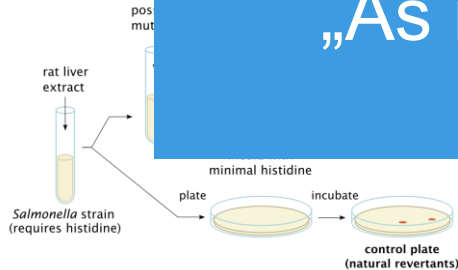


Table 2: TTC values – classification of substances

Classification	TTC value in $\mu\text{g}/\text{person per day}$
Worst Case Assumption	
Potential DNA-reactive mutagens and/or carcinogens	0.15
Organophosphates and carbamates	18
Cramer Class III	90
Cramer Class II	540
Cramer Class I	1,800

EFSA Scientific Committee. (2019). Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. EFSA Journal, 17(6), e05708.

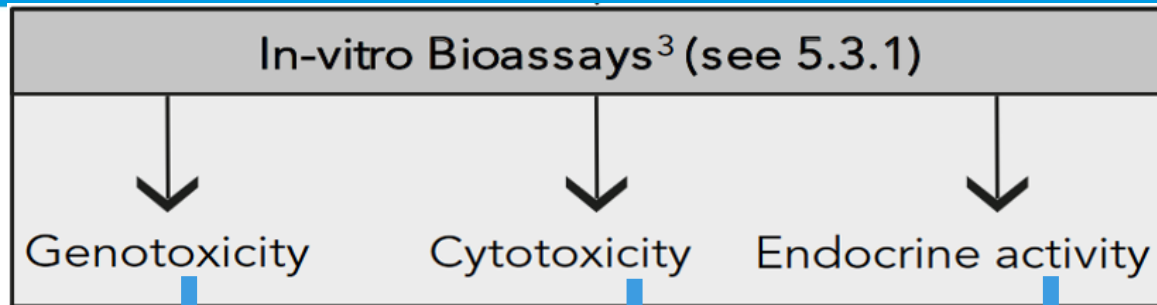
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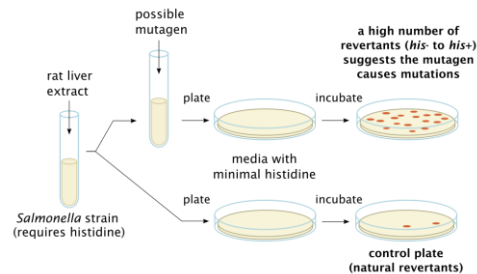
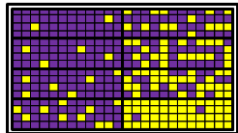
**600 x
higher**

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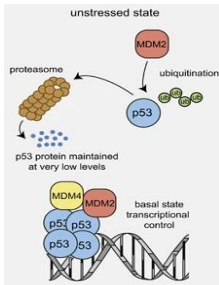
ILSI – NIAS report 2016 suggests bioassays for 3 endpoints!



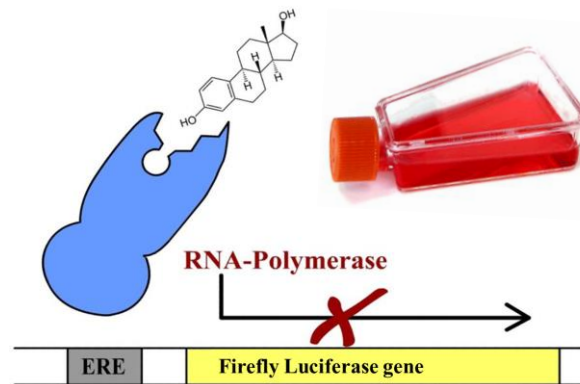
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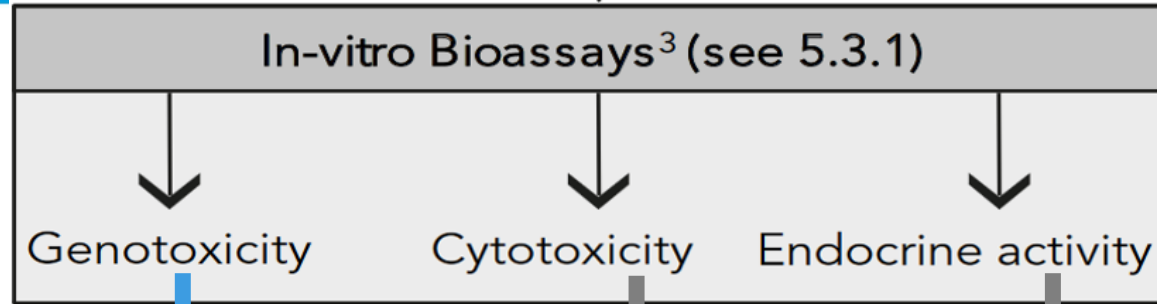
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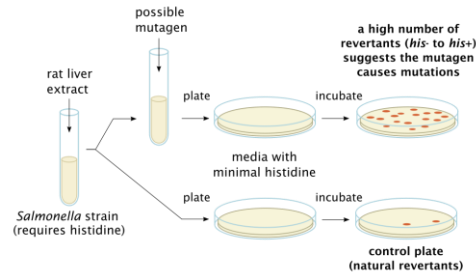
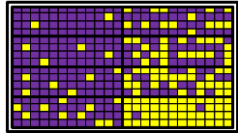
ER-CALUX, (Anti)AR-CALUX: Cell culture Test, includes Cytotoxicity



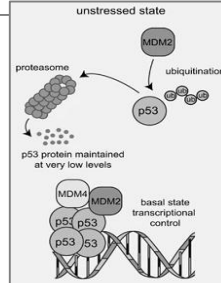
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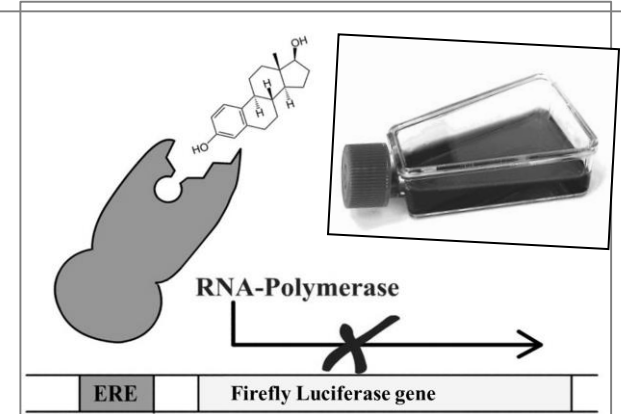
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HepG2 Assay, P53 CALUX, Micronucleus, High Content Screening: Cell Culture Tests for Chromosomal Damage



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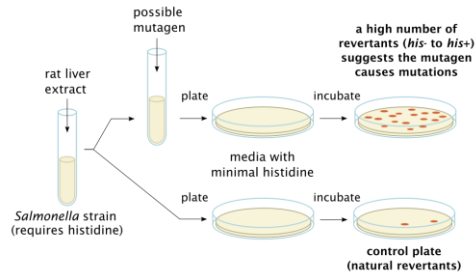
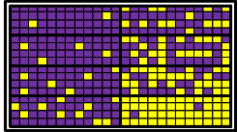
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In-vitro Bioassays³ (see 5.3.1)

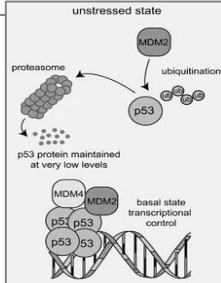
Genoto

Observation: Endocrine Activity strongly in focus again!

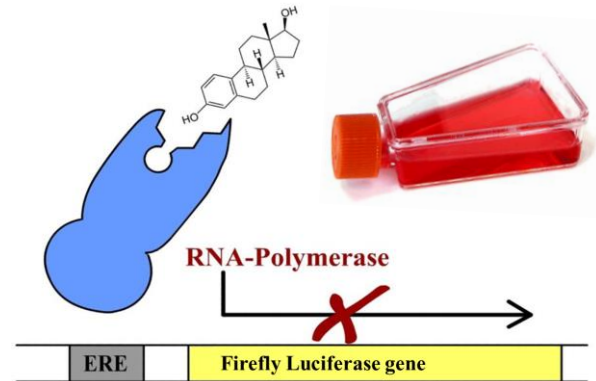
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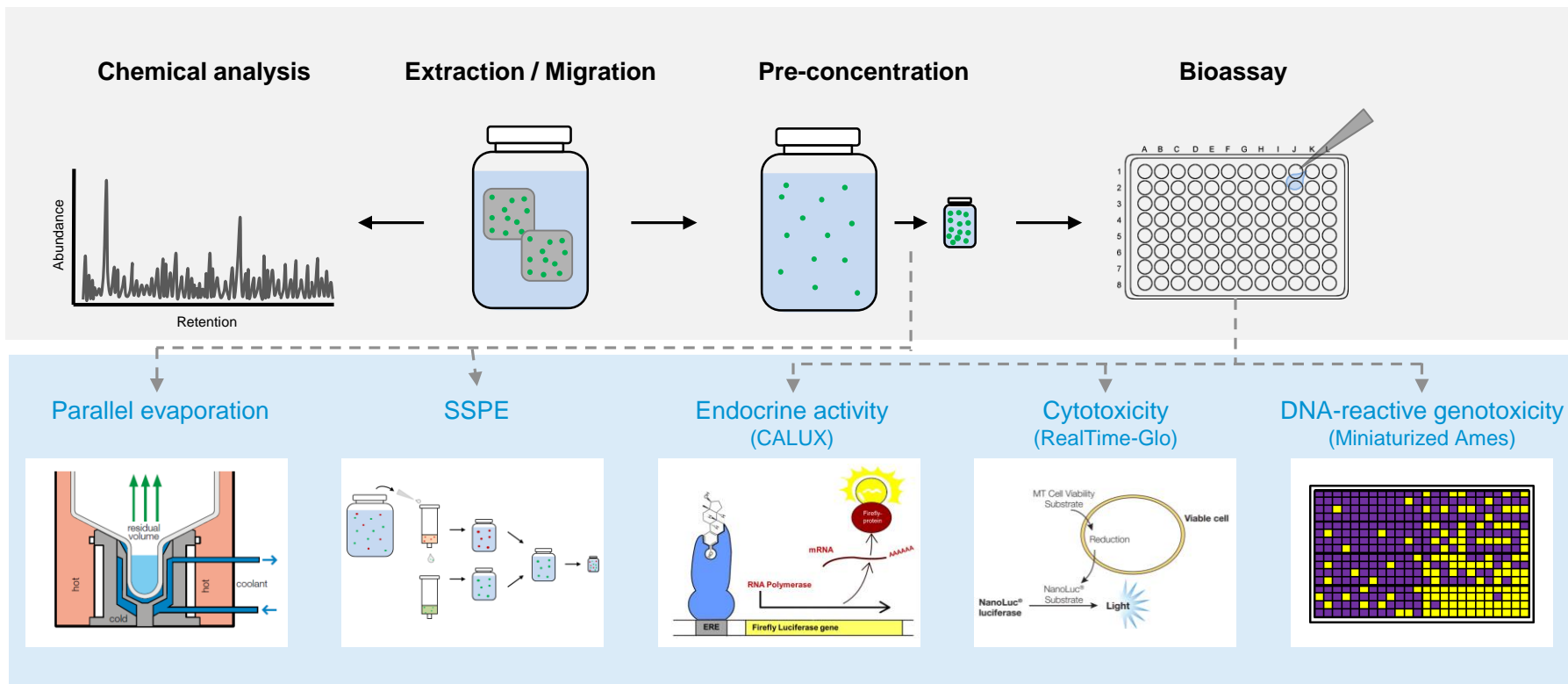
HepG2 Assay, P53 CALUX, Micronucleus, High Content Screening: Cell Culture Tests for Chromosomal Damage



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Workflow: How does a bioassay analysis work in practice?



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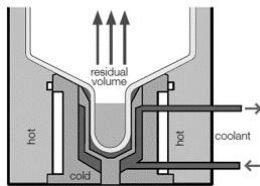
Evaluation of:

- 8 different genotoxicity tests
- > 60 model substances for genotox test development and validation

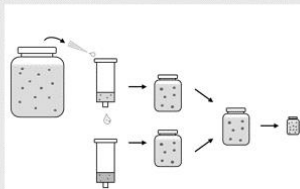
The Ames Test has the best detection limits of all Genotoxicity Bioassays

Detection Limit further improved by Miniaturization (approx. 5-fold)

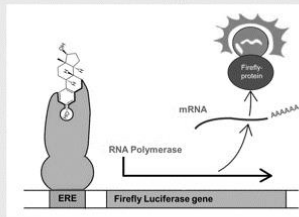
Parallel evaporation



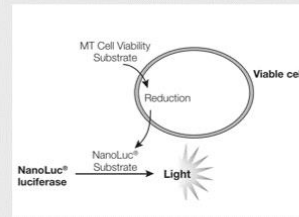
SSPE



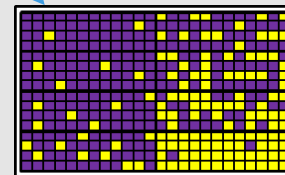
Endocrine activity
(CALUX)



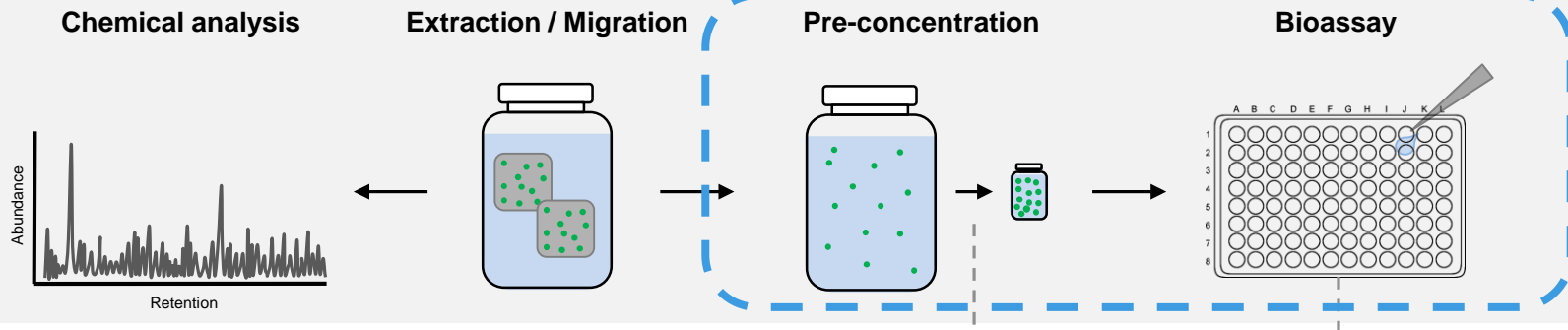
Cytotoxicity
(RealTime-Glo)



DNA-reactive genotoxicity
(Miniaturized Ames)

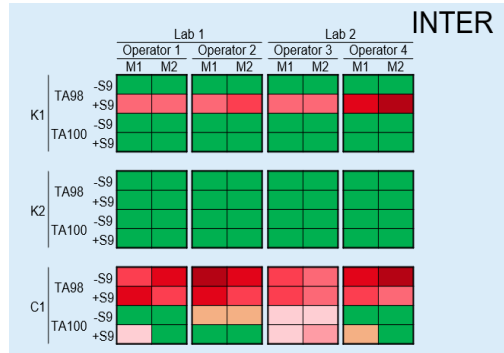


Workflow: How does a bioassay analysis work in practice?

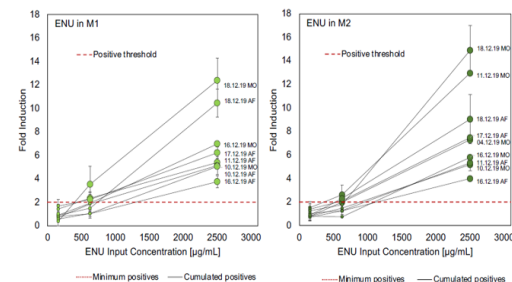


Validation of whole test method

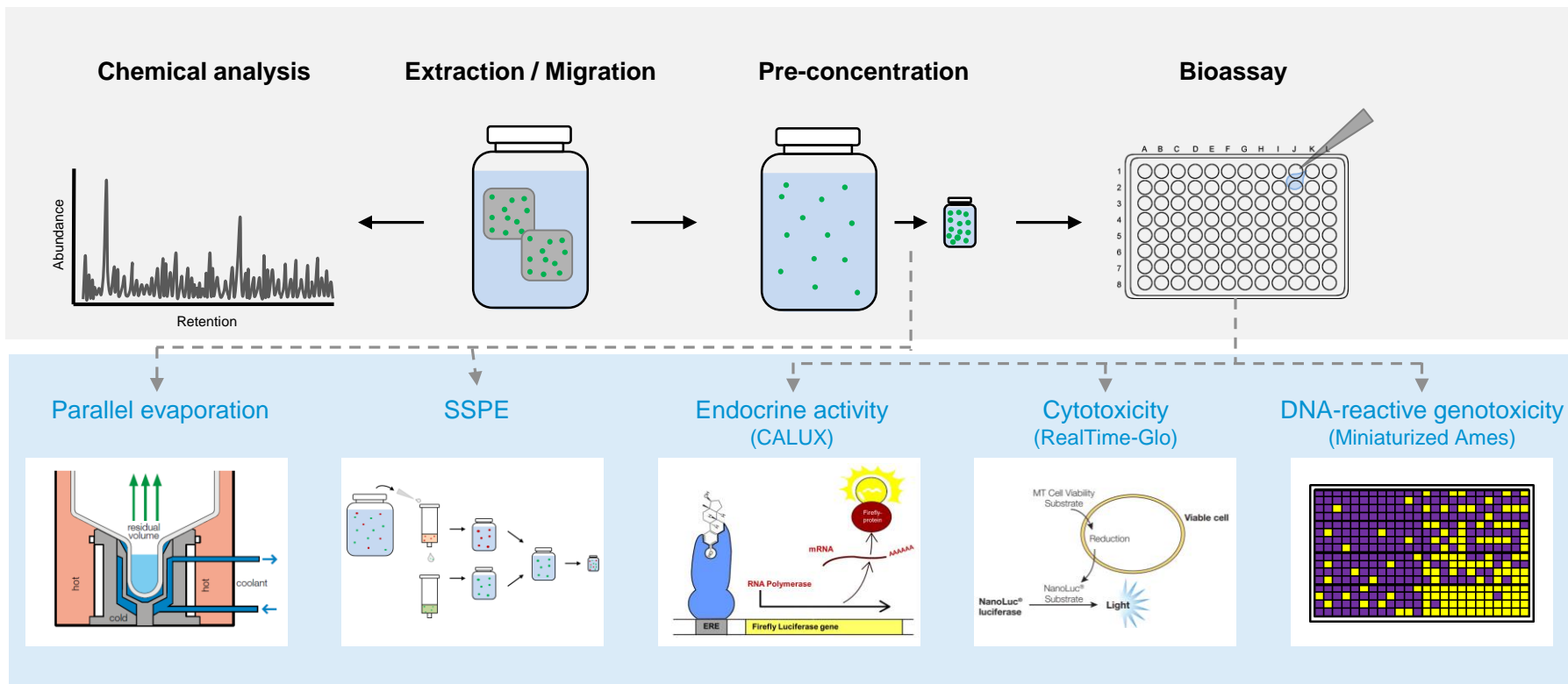
- In-house Validation at OFI based on requirements from ISO 17025
- Inter-Laboratory study at 3 Labs with FCM samples



INTRA



Workflow: How does a bioassay analysis work in practice?



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