# Agenda

**Industry board meeting, project "MIGRATOX"; December 6th, 2022, Vienna (Hybrid Event)**

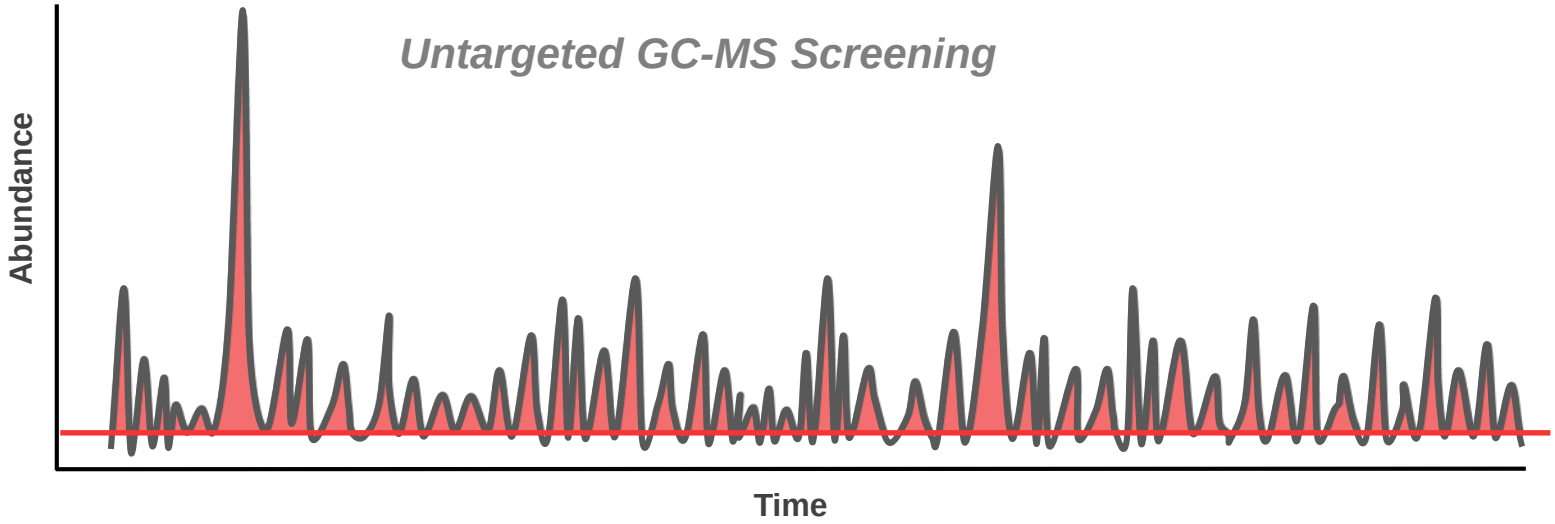
| | |
|---|---|
| 10:30 | Welcome and introduction |
| 10:45 | Christian Kirchnawy (OFI)<br>*"Migratox Project: What happened so far!"* |
| 11:00 | Maricel Marin-Kuan (Nestlé Research Centre)<br>*"Integrated multidisciplinary approach to support prioritization and packaging safety"* |
| 11:25 | B. Rainer & E. Haider (FH Campus Vienna)<br>*"Comparison of different Genotoxicity tests for selected Migratox FCM samples: […]"* |
| 11:50 | Open Discussion |
| 12:10 | Snacks & Networking |
| 13:10 | Christian Kirchnawy (OFI)<br>"*Updated Results from Screening >400 FCM samples: results for different materials: virgin/recycled, paper, polymers and coatings*" |
| 13:30 | Elisa Mayrhofer & Ida Peneder<br>"*Printing Inks as a potential risk factor for DNA-reactive substances: In-depth analysis of isolated fractions*" |
| 13:55 | Open Discussion |
| 14:10 | Thomas Czerny (FH Campus Vienna)<br>"*Ames Sense: Optimization of a miniaturized Ames test for better detection limits*" |
| 14:30 | Bernhard Rainer (University of Applied Sciences, Vienna)<br>"*Summary, Conclusions, Next Steps*" |
| 14:45 | Open Discussion, Opportunity to bring in your own ideas/wishes |
| 15:00 | Coffee & Networking |

# Anti-Trust Declaration

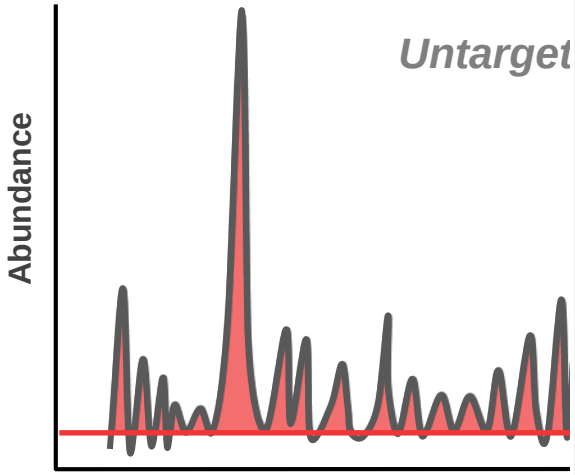
*“OFI and companies participating at OFI meetings shall not enter into any discussion, activity or conduct that may infringe, on its part or on the part of its members, any applicable competition law.*

*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.”*

# Main Project Goal: Risk Assessment for unidentified NIAS!



# Risk Assessment of unidentified NIAS




| Probenname: | MeOH Extrakt | Polarität: | Positiv | Medium: | MeOH |
|---|---|---|---|---|---|
| | C50H200N10O10S2P2 | | | | |
| Peakname | Summenformel | Score Summenformel | Masse (*m/z*) | Rt [Minuten] | Konzentration [ppb] |
| 114.0908 / 3.26 | C6H11NO | 60 | 114,0908 | 3,3 | 135,0 |
| 177.0657 / 3.32 | C9H8N2O2 | 90 | 177,0657 | 3,3 | 222,0 |
| 195.0875 / 3.92 | C8H10N4O2 | 84 | 195,0875 | 3,9 | 199,0 |
| 177.0656 / 3.94 | C9H8N2O2 | 65 | 177,0656 | 3,9 | 194,0 |
| 123.0626 / 3.94 | No formula found | 0 | 123,0626 | 4,0 | 157,0 |
| 122.0595 / 3.96 | C7H7NO | 52 | 122,0595 | 4,0 | 1209,0 |
| 135.0544 / 4.02 | C7H6N2O | 61 | 135,0544 | 4,0 | 149,0 |
| 151.0861 / 4.11 | C8H10N2O | 57 | 151,0861 | 4,1 | 202,0 |
| 120.0551 / 4.26 | No formula found | 0 | 120,0551 | 4,3 | 306,0 |
| 340.2594 / 4.35 | C18H33N3O3 | 90 | 340,2594 | 4,3 | 118,0 |
| 151.0865 / 4.80 | C8H10N2O | 63 | 151,0865 | 4,8 | 459,0 |
| 160.0867 / 4.82 | C9H9N3 | 84 | 160,0867 | 4,8 | 302,0 |
| 217.1072 / 5.02 | C10H16O5 | 46 | 217,1072 | 5,0 | 162,0 |
| 323.1707 / 5.00 | C14H26O8 | 83 | 323,1707 | 5,0 | 599,0 |
| 211.1437 / 5.24 | C11H18N2O2 | 59 | 211,1437 | 5,2 | 148,0 |
| 453.3436 / 5.24 | C24H44N4O4 | 71 | 453,3436 | 5,3 | 1419,0 |
| 275.1492 / 5.30 | C13H22O6 | 80 | 275,1492 | 5,3 | 286,0 |
| 237.0756 / 5.34 | C12H12O5 | 66 | 237,0757 | 5,4 | 169,0 |
| 246.1417 / 5.64 | C6H15N9O2 | 84 | 246,1417 | 5,5 | 263,0 |
| 245.1294 / 5.90 | C14H16N2O2 | 77 | 245,1294 | 5,5 | 1427,0 |
| 245.1386 / 5.47 | C12H20O5 | 88 | 245,1386 | 5,5 | 1427,0 |
| 188.0930 / 5.56 | C9H9N5 | 80 | 188,0930 | 5,6 | 324,0 |
| 281.1024 / 5.56 | C14H16O6 | 55 | 281,1024 | 5,6 | 156,0 |
| 149.0706 / 5.58 | C8H8N2O | 43 | 149,0706 | 5,6 | 384,0 |
| 251.1855 / 5.58 | C12H26O5 | 57 | 251,1855 | 5,6 | 644,0 |
| 207.0767 / 5.62 | C10H10N2O3 | 59 | 207,0767 | 5,6 | 217,0 |
| 235.1904 / 5.64 | C13H22N4 | 26 | 235,1904 | 5,6 | 128,0 |
| 180.1018 / 5.73 | C10H13NO2 | 64 | 180,1018 | 5,7 | 220,0 |
| 228.1597 / 5.77 | C12H21NO3 | 40 | 228,1597 | 5,8 | 185,0 |
| 566.4287 / 5.88 | C30H55N5O5 | 87 | 566,4287 | 5,9 | 309,0 |

# TTC concept – Risk assessment

**Table 2:** TTC values – classification of substances

| Classification | Worst Case Assumption | TTC value in µg/person per day |
|---|---|---|
| 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.*

# TTC concept – Risk assessment

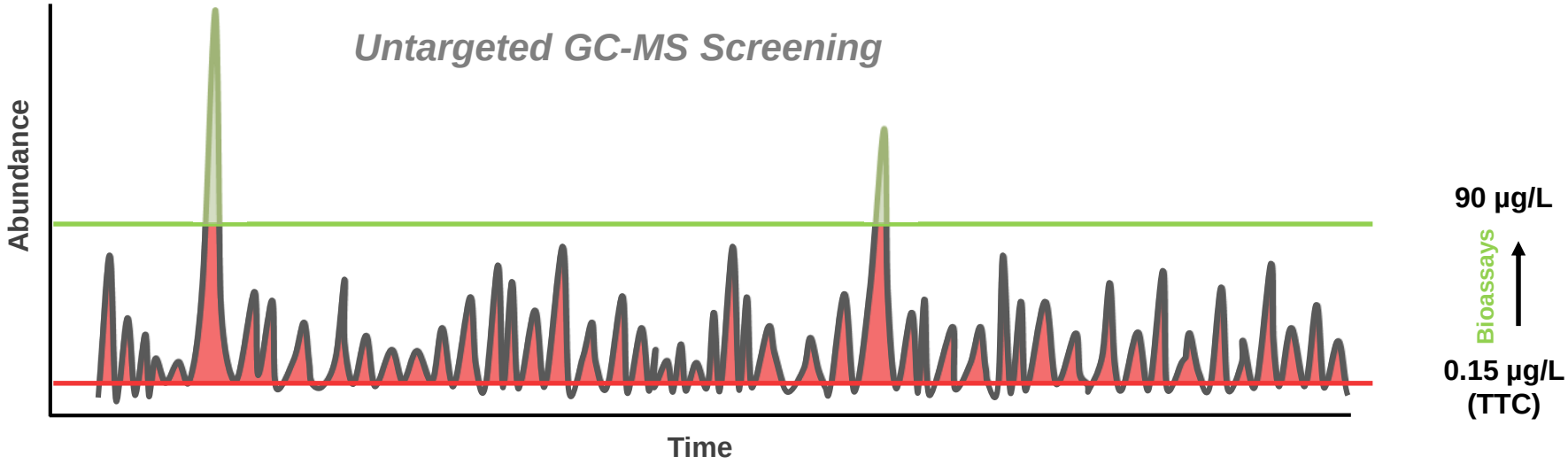
**Table 2:** TTC values – classification of substances

| Classification (Worst Case Assumption) | TTC value in µg/person per day |
|---|---|
| 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 |

**600 x higher**

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

# Risk Assessment of unidentified NIAS



# Original Idea in ILSI – NIAS Report (2016)

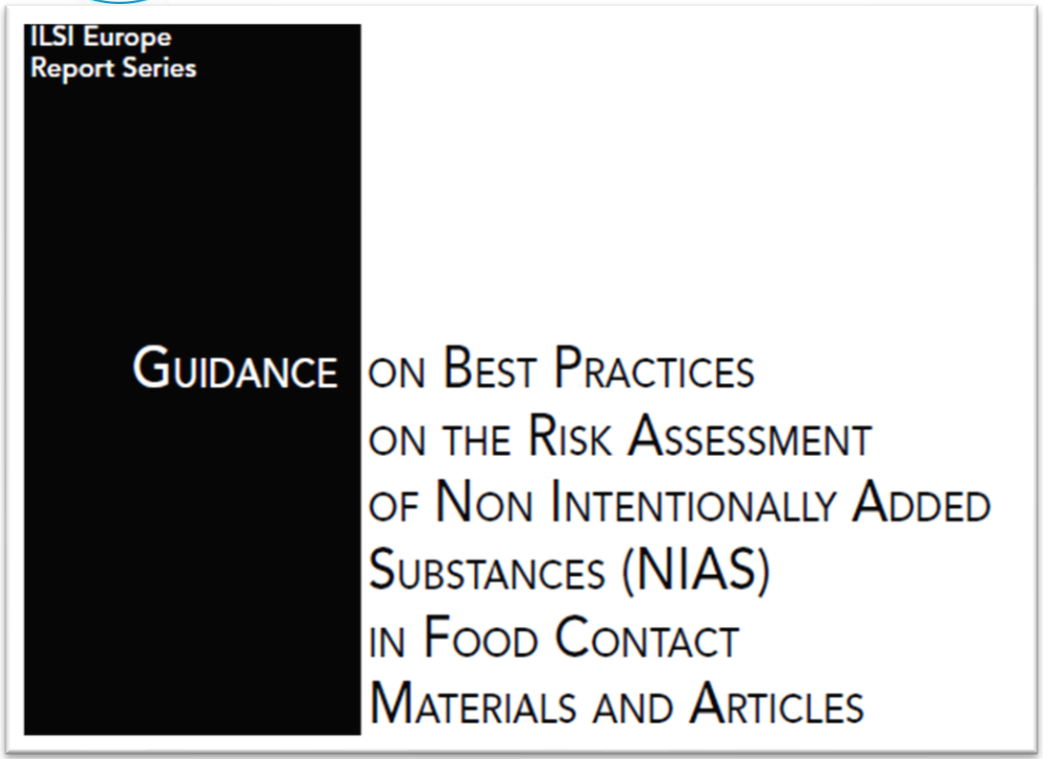
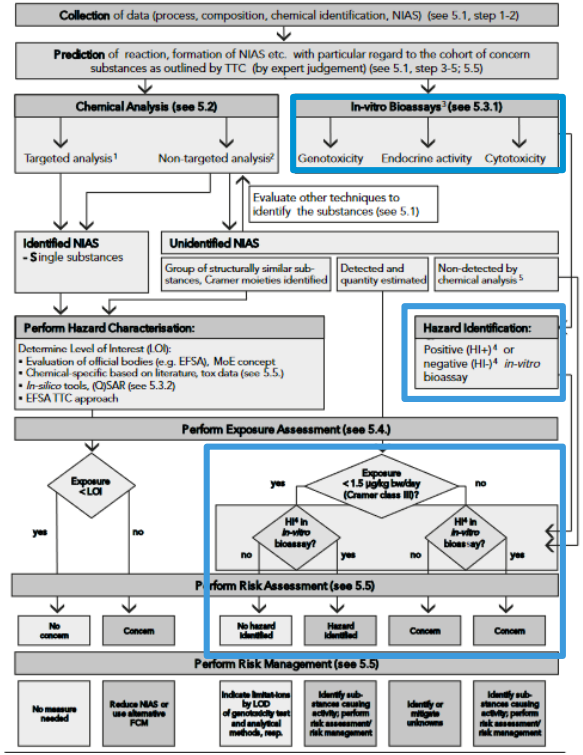



*Figure 1: Flowchart for the risk assessment of NIAS (may also apply to substances other than NIAS).*

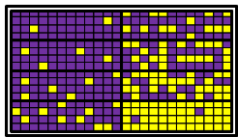


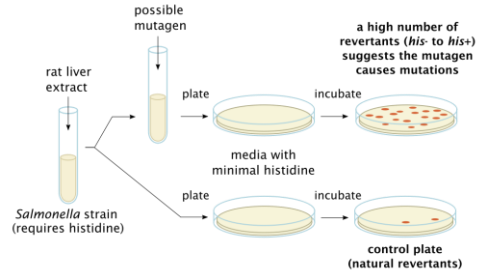
# ILSI – NIAS report 2016 suggests bioassays for 3 endpoints!

**In-vitro Bioassays[3] (see 5.3.1)**

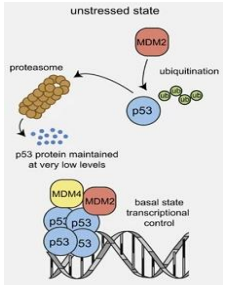
- Genotoxicity
- Cytotoxicity
- Endocrine activity

**Ames-Test:** Bacteria Test
DNA-reactive
Genotoxicity

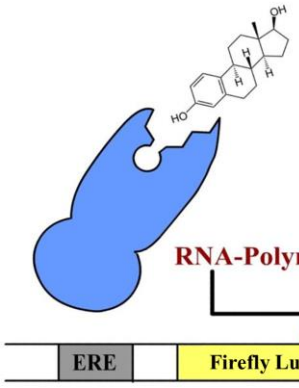





**HepG2 Assay, P53 CALUX, Micronucleus, High Content Screening:**
Cell Culture Tests for
Chromosomal Damage




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




# Project Goal: Selection of Bioassay Battery

**Major Project Goal in Project Migratox:**
„Comparison of different in-vitro bioassays and selection of suitable tests for a bioassay battery“

**Goal: Bioassay Battery as small as possible!**
„As many bioassays as necessary but as few as possible“

# TTC concept – Risk assessment

**Table 2:** TTC values – classification of substances

| Classification | Worst Case Assumption | TTC value in µg/person per day |
|---|---|---|
| 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 |

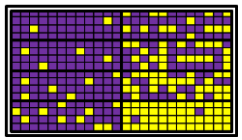
*EFSA Scientific Committee. (2019). Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. EFSA Journal, 17(6), e05708.*

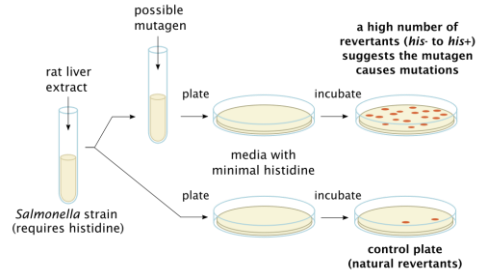
# ILSI – NIAS report 2016 suggests bioassays for 3 endpoints!

**In-vitro Bioassays[3] (see 5.3.1)**

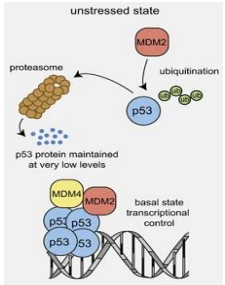
- Genotoxicity
- Cytotoxicity
- Endocrine activity

**Ames-Test:** Bacteria Test
DNA-reactive
Genotoxicity

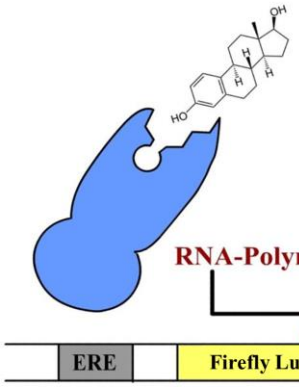





**HepG2 Assay, P53 CALUX, Micronucleus, High Content Screening:**
Cell Culture Tests for
Chromosomal Damage




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

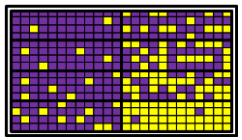


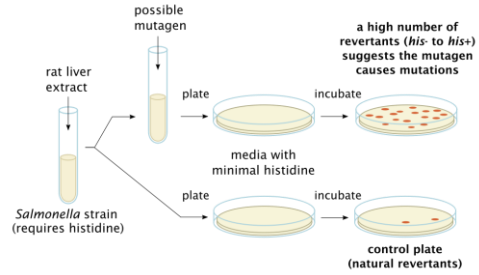

# ILSI – NIAS report 2016 suggests bioassays for 3 endpoints!

**In-vitro Bioassays[3] (see 5.3.1)**

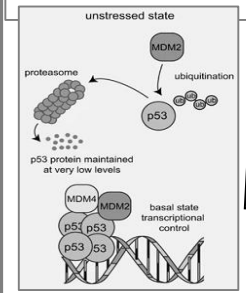
- Genotoxicity
- Cytotoxicity
- Endocrine activity

**Ames-Test:** Bacteria Test
DNA-reactive
Genotoxicity

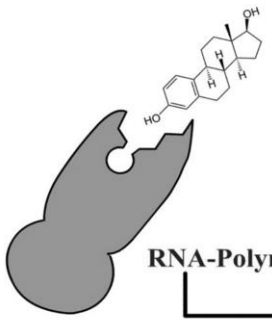


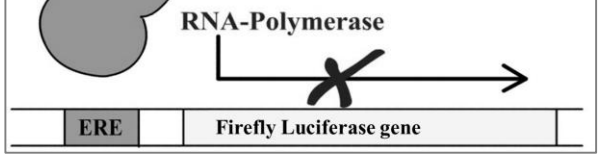



**HepG2 Assay, P53 CALUX, Micronucleus, High Content Screening:**
Cell Culture Tests for Chromosomal Damage




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



# Planned working steps (original Slide from Kick-Off)

- Sensitivity:
  - comparison of *in-vitro* methods / sample preparations
  - representative test substances for comparing sensitivity
- Sample preparation:
  - Comparison of sample preparation methods
  - Validation: loss of volatiles, contaminations,..
- Validation:
  - Ensure that methods are suitable for FCM migrates/extracts
- Standardization
  - Defined protocols, specific guidelines
  - Acceptance by cooperation with authorities
- Sample Screening

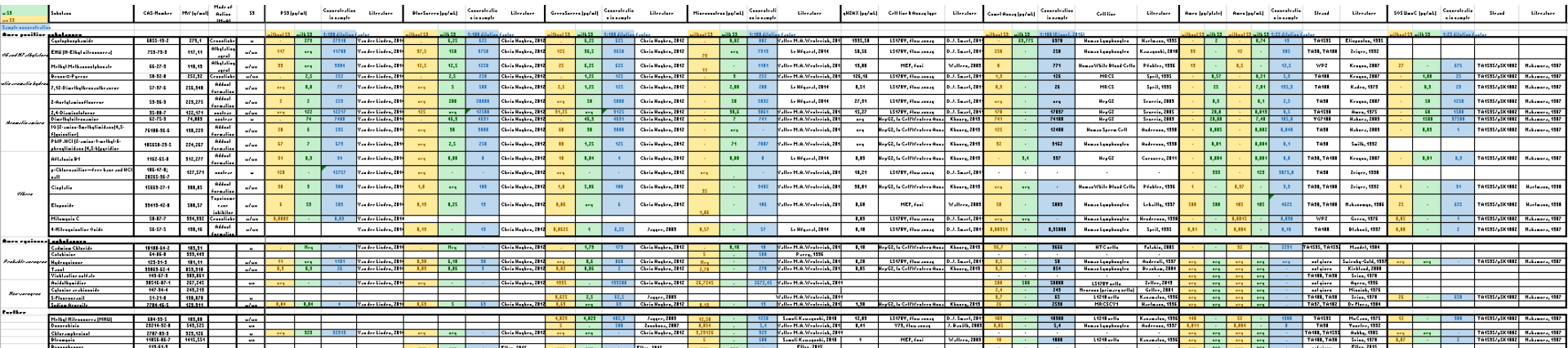
# Sensitivity of 8 different existing genotoxicity tests compared

Evaluation of:

- 8 different gentoxicity tests
- 30 model substances for genotox test development and validation (ECVAM)
- 36 packaging relevant substances

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

**BUT: Most substances cannot be detected at the low 0.15 µg/day limit!**



# Detection limits strongly improved by miniaturization

**Evaluation of the Detection limits of genotoxicity tests**

Rainer et al., 2018

Food Additives & Contaminants: Part A

ISSN: 1944-0049 (Print) 1944-0057 (Online) Journal homepage: http://www.tandfonline.com/loi/tfac20

Suitability of the Ames test to characterise genotoxicity of food contact material migrates

Bernhard Rainer, Elisabeth Pinter, Thomas Czerny, Elisabeth Riegel, Christian Kirchnawy, Maricel Marin-Kuan, Benoît Schilter & Manfred Tacker

Pinter et al., 2020

foods

MDPI

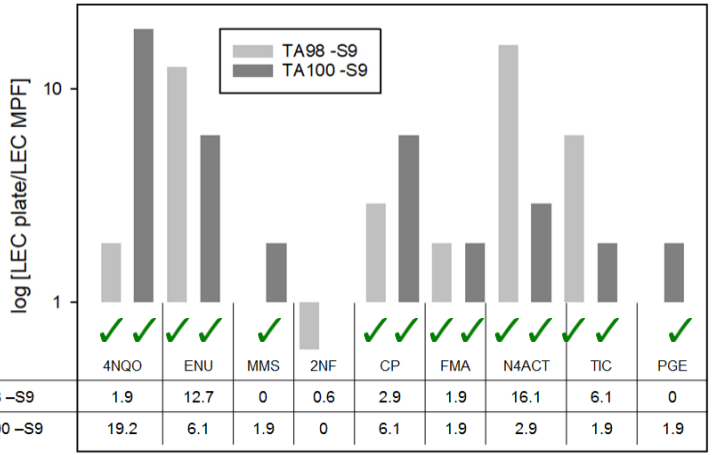
Review

Evaluation of the Suitability of Mammalian *In Vitro* Assays to Assess the Genotoxic Potential of Food Contact Materials

Elisabeth Pinter [1,*], Bernhard Rainer [1], Thomas Czerny [1], Elisabeth Riegel [1], Benoît Schilter [2], Maricel Marin-Kuan [2] and Manfred Tacker [1]

**Approx. 6-fold Improvement of detection limits by miniaturization**

Rainer et al., 2021




| | 4NQO | ENU | MMS | 2NF | CP | FMA | N4ACT | TIC | PGE |
|---|---|---|---|---|---|---|---|---|---|
| TA98 –S9 | 1.9 | 12.7 | 0 | 0.6 | 2.9 | 1.9 | 16.1 | 6.1 | 0 |
| TA100 –S9 | 19.2 | 6.1 | 1.9 | 0 | 6.1 | 1.9 | 2.9 | 1.9 | 1.9 |

The Ames test has better detection limits than mammalian genotoxicity assays.
Using the Ames MPF format the sensitivity can be further improved.

# Further Improvement of Detection Limits

- **Ames Sense:** Optimized miniaturized Ames test with lower sample requirement and improved detection limits
  - Work in Progress, Update today

- **Combination with Fractionation:** Thin layer chromatography, SPE

- **Sample Preparation:** Up to 2.000-fold concentration

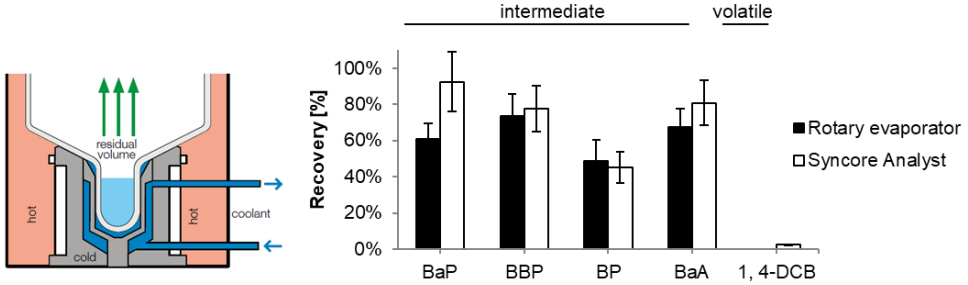
# Planned working steps (original Slide from Kick-Off)

- Sensitivity:
  - comparison of *in-vitro* methods / sample preparations
  - representative test substances for comparing sensitivity
- Sample preparation:
  - Comparison of sample preparation methods
  - Validation: loss of volatiles, contaminations,..
- Validation:
  - Ensure that methods are suitable for FCM migrates/extracts
- Standardization
  - Defined protocols, specific guidelines
  - Acceptance by cooperation with authorities
- Sample Screening

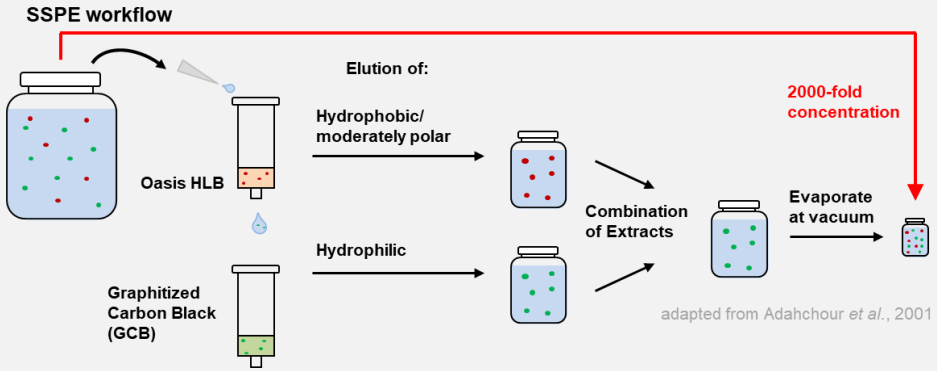
# Milestones: Development of sample preparation strategy

Up to 2000 fold up-concentration with validated method!

**95% ethanol:**
Fully automated evaporation procedure




**50-10% ethanol, water and 3% acetic acid:**
Sequential solid phase extraction

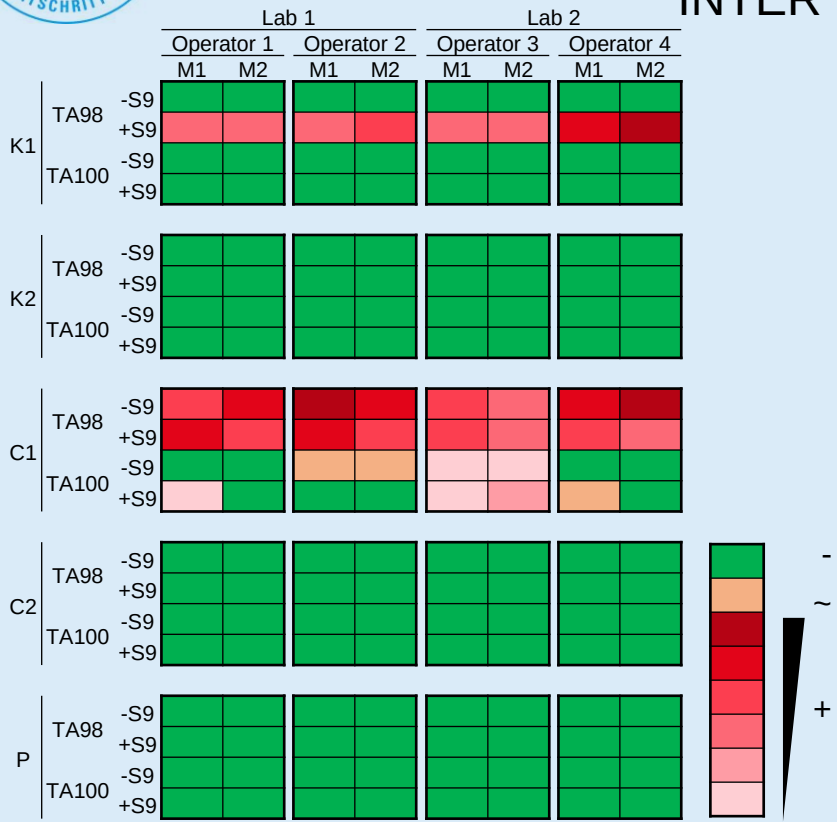


# Planned working steps (original Slide from Kick-Off)

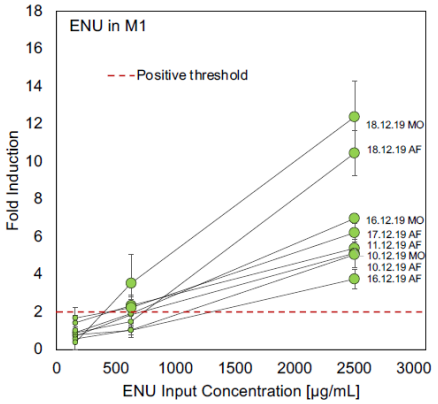
- Sensitivity:
  - comparison of *in-vitro* methods / sample preparations
  - representative test substances for comparing sensitivity
- Sample preparation:
  - Comparison of sample preparation methods
  - Validation: loss of volatiles, contaminations,..
- Validation:
  - Ensure that methods are suitable for FCM migrates/extracts
- Standardization
  - Defined protocols, specific guidelines
  - Acceptance by cooperation with authorities
- Sample Screening

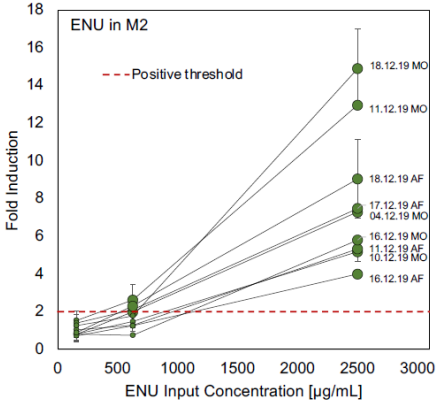
# Milestones: Intra- and interlab validation of Ames MPF

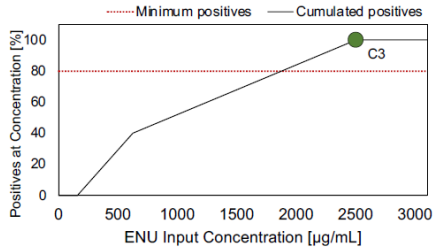
## INTER

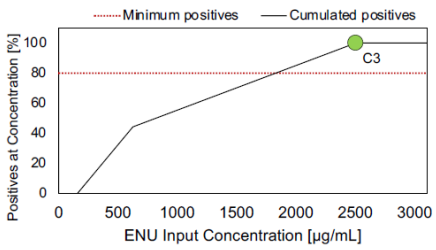



## INTRA



# Planned working steps (original Slide from Kick-Off)

- Sensitivity:
  - comparison of *in-vitro* methods / sample preparations
  - representative test substances for comparing sensitivity
- Sample preparation:
  - Comparison of sample preparation methods
  - Validation: loss of volatiles, contaminations,..
- Validation:
  - Ensure that methods are suitable for FCM migrates/extracts
- Standardization
  - Defined protocols, specific guidelines
  - Acceptance by cooperation with authorities
- Sample Screening

# ILSI Europe Expert Group also recommends Ames Test

ILSI Europe

**Value and limitation of *in vitro* bioassays to support the application of the threshold of toxicological concern to prioritise unidentified chemicals in food contact materials**

Benoit Schilter, Karin Burnett, Chantra Eskes, **Lucie Geurts**, Mélanie Jacquet, Christian Kirchnawy, ...show all

Pages 1903-1936 | Received 14 May 2019, Accepted 30 Aug 2019, Published online: 24 Sep 2019

Download citation https://doi.org/10.1080/19440049.2019.1664772 Check for updates

**Conclusions:**

- Focus on Ames-Test (as stand-alone bioassay)
- Combination with data from:
  - Chemical Analysis
  - Formulation Data
- **If all lines of evidence consistently show no sign of DNA-reactive genotoxic substances**
  **→ Cramer Class III**

| Expert | Affilation |
|---|---|
| Benoit Schilter (Chair) | Nestlé |
| Alan Boobis | Imperial College London |
| Karin Burnett | Consultant |
| Chantra Eskes | Europ. Society of Toxicology In Vitro |
| Mélanie Jacquet | Danone |
| Peter Oldring | The Valspar Company |
| Gabriele Pieper | Tetra Pak |
| Manfred Tacker | University of Applied Sciences |
| Heinz Traussnig | Mayr-Melnhof Karton |
| Peter van Herwijnen | Dow Europe |
| Lucie Geurts | ILSI Europe |
| Elisabeth Pinter | University of Applied Sciences |
| Christian Kirchnawy | OFI |

# ILSI Europe Expert Group also recommends Ames Test

ILSI Europe

**Value and limitation of *in vitro* bioassays to support the application of the threshold of toxicological concern to prioritise unidentified chemicals in food contact materials**

Benoit Schilter, Karin Burnett, Chantra Eskes, **Lucie Geurts**, Mélanie Jacquet, Christian Kirchnawy, ...show all

Pages 1903-1936 | Received 14 May 2019, Accepted 30 Aug 2019, Published online: 24 Sep 2019

Download citation https://doi.org/10.1080/19440049.2019.1664772 Check for updates

**Conclusions:**

- Focus on Ames-Test (as stand-alone bioassay)
- Combination with data from:
  - Chemical Analysis
  - Formulation Data
- **If all lines of evidence consistently show no sign of DNA-reactive genotoxic substances**
  **→ Cramer Class III**

| Expert | Affilation |
|---|---|
| **Benoit Schilter (Chair)** | **Nestlé** |
| Alan Boobis | Imperial College London |
| Karin Burnett | Consultant |
| Chantra Eskes | Europ. Society of Toxicology In Vitro |
| **Mélanie Jacquet** | **Danone** |
| **Peter Oldring** | **The Valspar Company** |
| Gabriele Pieper | Tetra Pak |
| **Manfred Tacker** | **University of Applied Sciences** |
| **Heinz Traussnig** | **Mayr-Melnhof Karton** |
| Peter van Herwijnen | Dow Europe |
| Lucie Geurts | ILSI Europe |
| **Elisabeth Pinter** | **University of Applied Sciences** |
| **Christian Kirchnawy** | **OFI** |

# Planned working steps (original Slide from Kick-Off)

- Sensitivity:
  - comparison of *in-vitro* methods / sample preparations
  - representative test substances for comparing sensitivity
- Sample preparation:
  - Comparison of sample preparation methods
  - Validation: loss of volatiles, contaminations,..
- Validation:
  - Ensure that methods are suitable for FCM migrates/extracts
- Standardization
  - Defined protocols, specific guidelines
  - Acceptance by cooperation with authorities
- Sample Screening: Overview in my next presentation!

# Agenda

**Industry board meeting, project "MIGRATOX"; December 6th, 2022, Vienna (Hybrid Event)**

| | |
|---|---|
| 10:30 | Welcome and introduction |
| 10:45 | Christian Kirchnawy (OFI)<br>*"Migratox Project: What happened so far!"* |
| 11:00 | Maricel Marin-Kuan (Nestlé Research Centre)<br>*"Integrated multidisciplinary approach to support prioritization and packaging safety"* |
| 11:25 | B. Rainer & E. Haider (FH Campus Vienna)<br>*"Comparison of different Genotoxicity tests for selected Migratox FCM samples: […]"* |
| 11:50 | Open Discussion |
| 12:10 | Snacks & Networking |
| 13:10 | Christian Kirchnawy (OFI)<br>"*Updated Results from Screening >400 FCM samples: results for different materials: virgin/recycled, paper, polymers and coatings*" |
| 13:30 | Elisa Mayrhofer & Ida Peneder<br>"*Printing Inks as a potential risk factor for DNA-reactive substances: In-depth analysis of isolated fractions*" |
| 13:55 | Open Discussion |
| 14:10 | Thomas Czerny (FH Campus Vienna)<br>"*Ames Sense: Optimization of a miniaturized Ames test for better detection limits*" |
| 14:30 | Bernhard Rainer (University of Applied Sciences, Vienna)<br>"*Summary, Conclusions, Next Steps*" |
| 14:45 | Open Discussion, Opportunity to bring in your own ideas/wishes |
| 15:00 | Coffee & Networking |