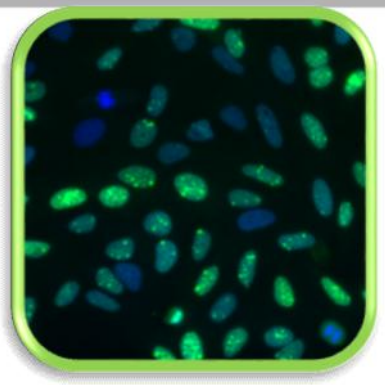

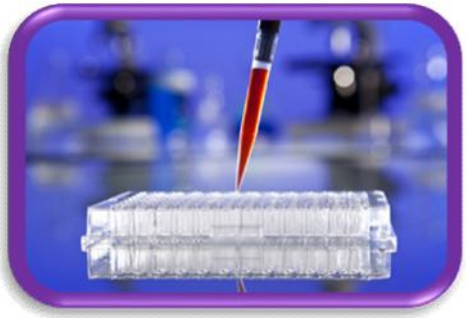

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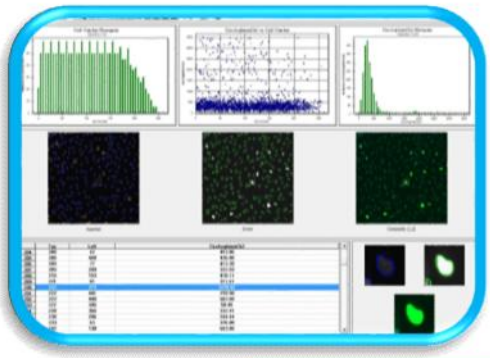
Bioassays and Packaging Safety



Maricel Marin Kuan,
maricel.marin-kuan@rdls.nestle.com
Chemical Food Safety group,
Nestlé Research Center, Lausanne, Switzerland

BioDetectors Conference, Naples 6-7 April 2017

Safety of plastics & packaging in general under scrutiny, lots of media attention for endocrine activity

Science News … *from universities, journals, and other research organizations*

Hormone-Mimics In Plastic Water Bottles Act As Functional Estrogens

ScienceDaily (Mar. 27, 2009) — Plastic packaging is not without its downsides, and if you thought mineral water was 'clean', it may be time to think again. According to Martin Wagner and Jörg Oehlmann from the Department of Aquatic Ecotoxicology at the Goethe University in Frankfurt am Main, Germany, plastic mineral water bottles contaminate drinking water with estrogenic chemicals.

BPA's Dangerous Chemical Cousin

January 13, 2011 | Sarah Mosko | chemicals, BPA, bisphenol a, hormones, hormone, estrogen, bpaf, bisphenol af

It would have been hard to get through 2010 without bumping into some scary information about the plastic ingredient Bisphenol A, aka BPA, like the fact it leaches from polycarbonate baby bottles and…

Continue Reading »

New Warnings about BPA

November 29, 2010 | Brita Belli | Daily News | chemicals, BPA, bisphenol a, endocrine, hormones, frederick vom saal, plastic, reproduction

An interview on the environmental website Yale360 raises renewed concerns about the health dangers of bisphenol-A, or BPA, a chemical found in polycarbonate plastic, in the epoxy resins lining most canned food, in dental sealants and on cash register receipts.

Continue Reading »

Environmental Hormones in Food Packaging: Migration into Food and the Environment

Jane Muncke, PhD

…more than 50 known or suspected endocrine disrupters currently are legally used in food packaging materials… These substances have been authorized by food safety agencies in the US (FDA, Food and Drug Administration) and/or EU (EFSA, European Food Safety Authority). Authorization is based on toxicological testing that mainly targets carcinogens but does not explicitly include hormone mimicking toxic mechanisms…

BPA phase-out

?

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Challenges to overcome in order to address FCM safety

How to address the safety of new BPA-alternatives intended to be applied to diverse food packaging materials

- More focus is needed on the finished materials and articles, including the manufacturing process used.
- Substances used in the manufacturing process may contain:
  - impurities
  - Reaction and degradation products can be formed (e.g oligomers)

It's necessary to evaluate the safety of all migrating substances

Scientific opinion EFSA 2016

Potential impact on the safety of substances used in food contact materials: updating

Scientific opinion EFSA 2016

1. Identity of the substance
2. Physical and chemical properties of migrating substances
3. Intended application of the substance and the food contact material
4. Data migration
   - Modelling
   - Simulation
   - Direct measurement in foods
5. Exposure of the consumer
   Levels of consumption of packaged foodstaffs
   Calculation of the exposure to set the toxicological data requirements
6. **Toxicity data**
   a) General considerations
   b) Toxicity testing of substances migrating from food contact material (Used and Non-intentionally added (including oligomers)
   c) For NIAS further genotoxicity considerations are needed
   d) General Toxicity considerations (e.g. neurotox, endocrine or immunological effects)
   e) Read-across
   f) Nanomaterial
   g) Toxicological assessment of polymeric additives and oligomers
   h) Toxicological assessment of impurities, reaction and degradation products (other than oligomers)

Risk assessment of packaging development : focus is complex

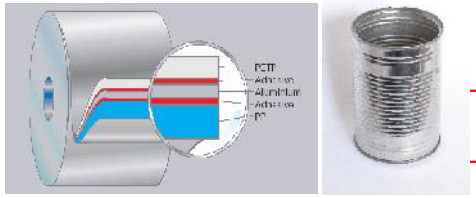
Chemical Industry

- Monomers
- Antioxidants
- Plasticizers
- Colorants
- Pigments
- Solvents
- UV stabilisers
- Slip agents
- Re...

Base Material Industries

- Plastics
- Rubbers
- Silicones
- Coatings
- Metals
- Paper & board
- Gla...
- In...
- Adhesives...

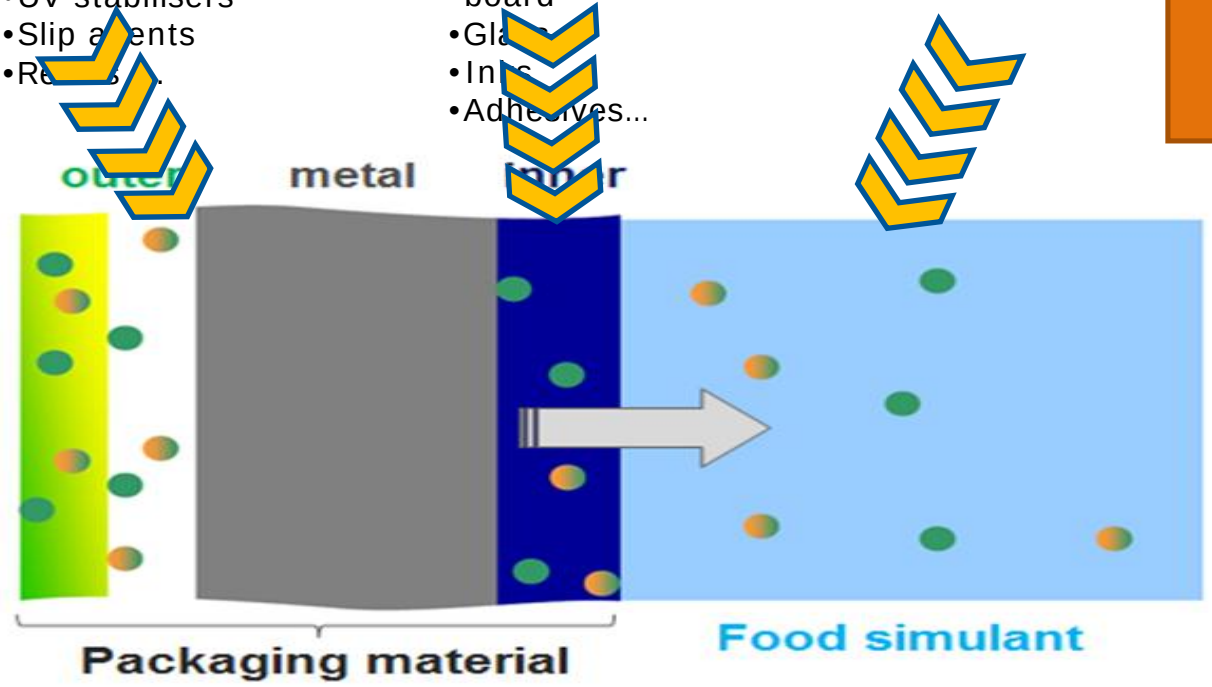
Converters



IAS

NIAS: mainly oligomers

Consumers Exposure???



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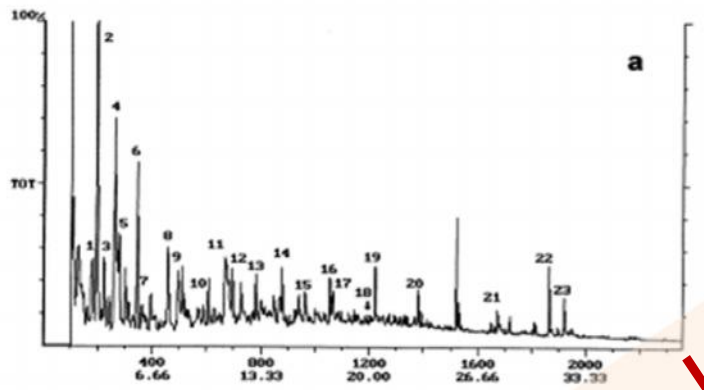
What is the problem?



GC-MS; LC-MS

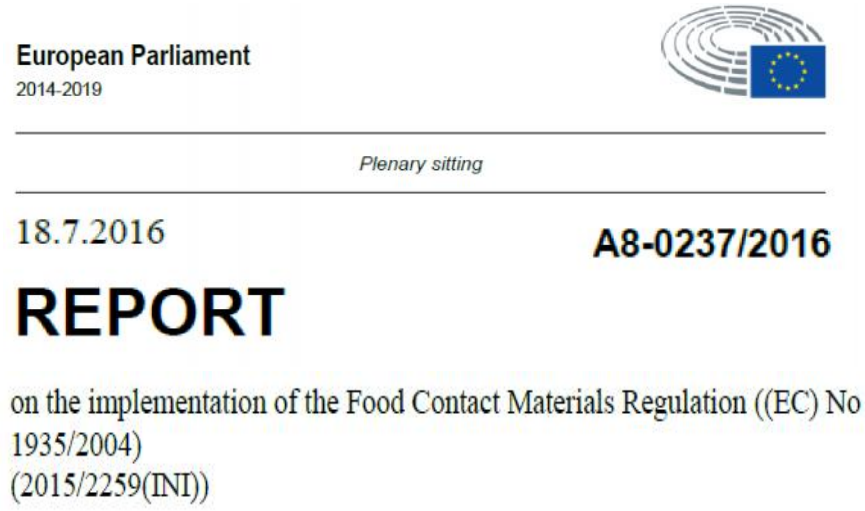
- **Partially characterized mixture of chemicals:**
  - Structurally characterized (identified)
  - Partially characterized
  - Not characterized/identified

- Identification
- Quantification
- Migration conditions
- Product applications
- toxicology

- Not feasible

Is there a role for bioassays in safety assessment of such mixtures?

Need to improve safety assessment of food contact materials (FCM)



European Parliament
2014-2019

Plenary sitting

18.7.2016 **A8-0237/2016**

REPORT

on the implementation of the Food Contact Materials Regulation ((EC) No 1935/2004)
(2015/2259(INI))

- Current paradigm for safety evaluation of FCMs is insufficient
- NIAS are especially mentioned
- Focus risk assessment on finished packaging
- Biotesting should be encouraged…
- …..

Biotesting should be encouranged as an optional premonitory measure → Safety of chemically complex FCM

Research on the development of both analytical and toxicological testing → Robust and cost-effective safety assessment.

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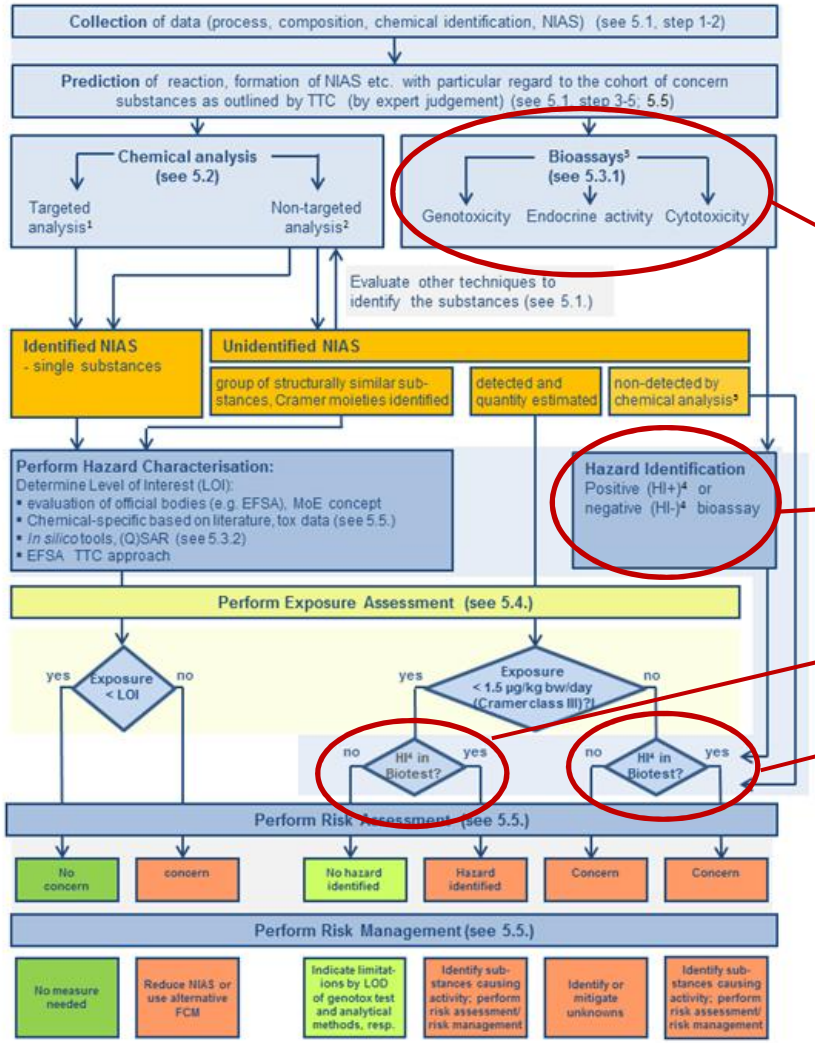
New approaches for safety assesment of FCM do mention bioassays



ILSI Europe
International Life Sciences Institute

Bioassays
Biotests
In vitro bioassays
Cell culture systems
….

From:
Guidance on best practices on the risk assesment of Non intentionally added substances (NIAS) in food contact materials

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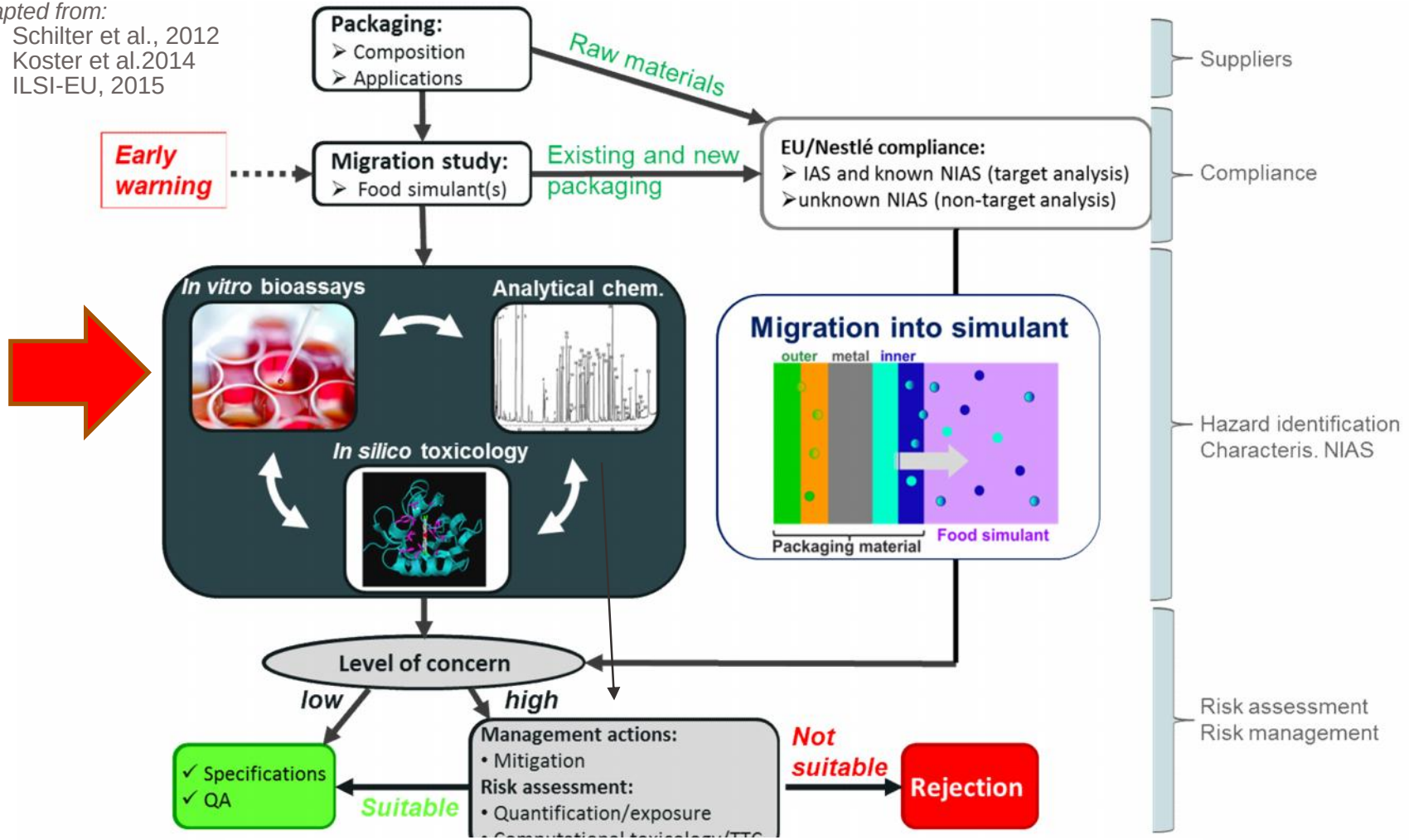
The Nestlé R&D approach

Adapted from:
- Schilter et al., 2012
- Koster et al.2014
- ILSI-EU, 2015



Packaging Sample Preparation Steps for Bioassays «one example»

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Extraction

1 dm2

H/A
10 ml

H/A
0.5 ml

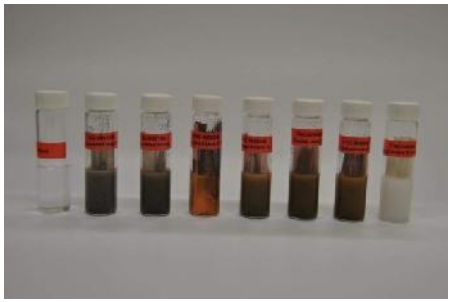
H/A
100
µL

H/A
400
µL

DMS
O
400
µL

SCREENIN
G GC-MS

BIOASSAYS



1 Blank
3 Replicates

Migration

1 Blank
3 Replicates

1
dm2

EtOH 95%
100 ml

EtOH 95%
0.5 ml

H/A
100 µL

H/A
400 µL

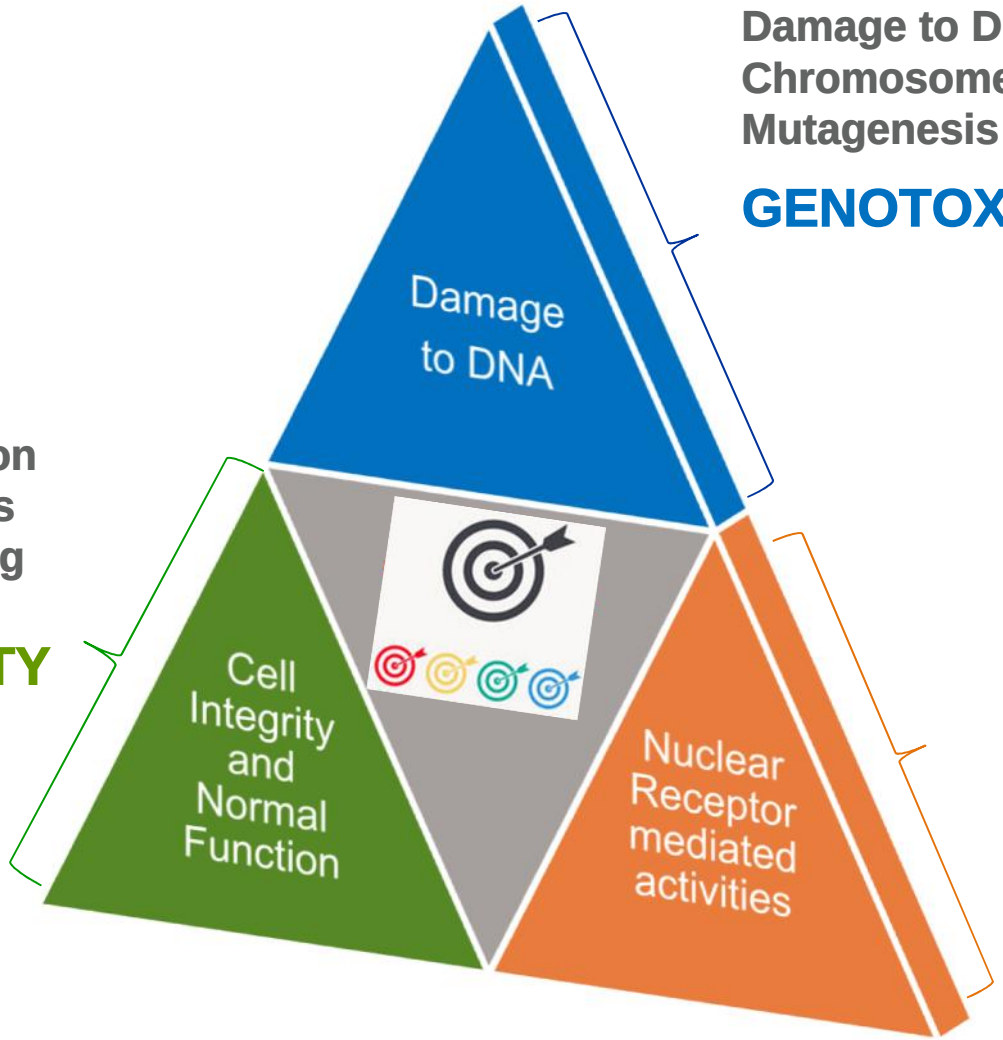
DMSO
400 µL

SCREENIN
G GC-MS

BIOASS
AYS

- H/A : Hexane/Acetone *- Extr. : Extraction* *- Mig. : Migration* *- R1: Replicate 1*

BIODETECTION APPROACH



Damage to DNA
Chromosome Breakage
Mutagenesis

GENOTOXICITY

Energy Production
Protein Synthesis
Cellular Recycling

CYTOTOXICITY

Hormone Mimicry
Altered Cellular Signaling
Metabolic Regulation

NUCLEAR RECEPTOR ACTIVATION

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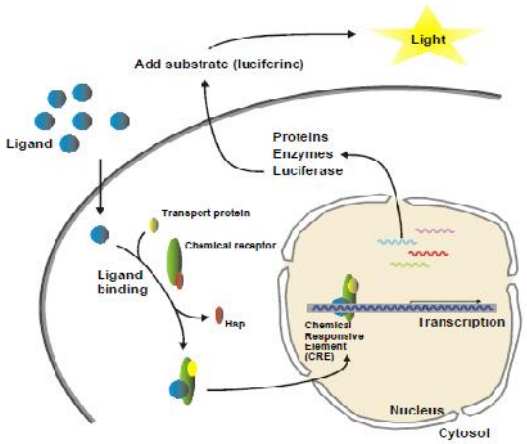
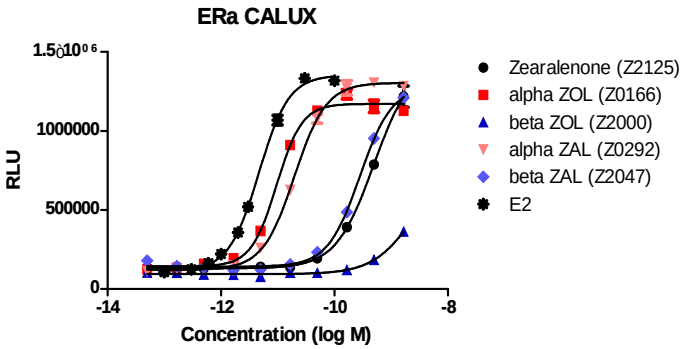
Nuclear Receptor Binding Activity

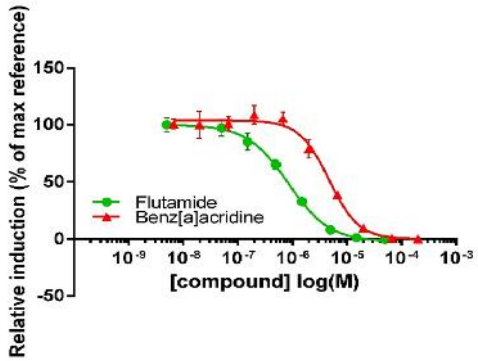
- Transcription activation **CALUX** bioassays: receptor mediated **C**hemically **A**ctivated **LU**ciferase e**X**pression



Receptor tested	Cell line	Reference compound	Activity tested
AhR	H4IIE	BaP	Agonist
ERα, ERβ	U2OS	17β-E_2	
AR		DHT	
Anti-ER		Tamoxifen	Antagonist
Anti-AR		Flutamide	
Nrf2		Coumarin	Induction



Agonist (mimic)



Antagonist (inhibit)

BioDetection Systems
CALUX®

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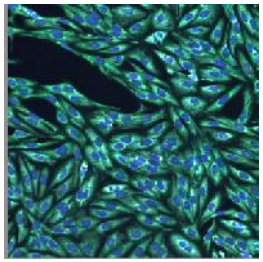
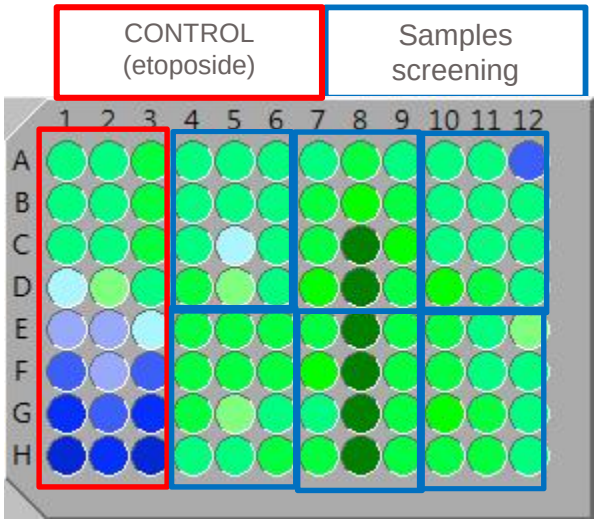
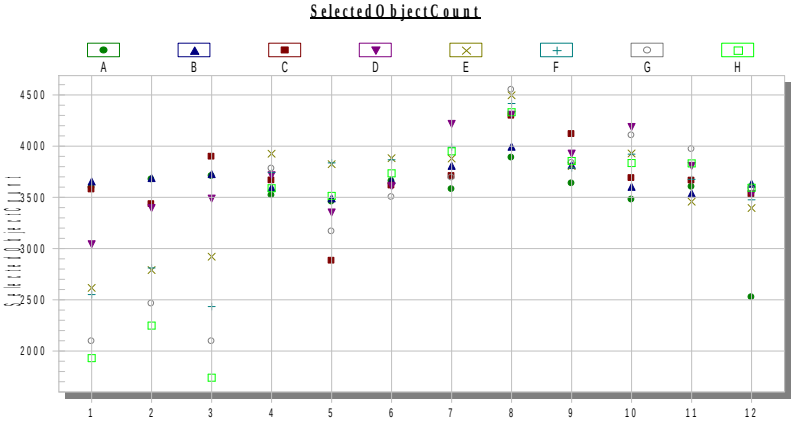
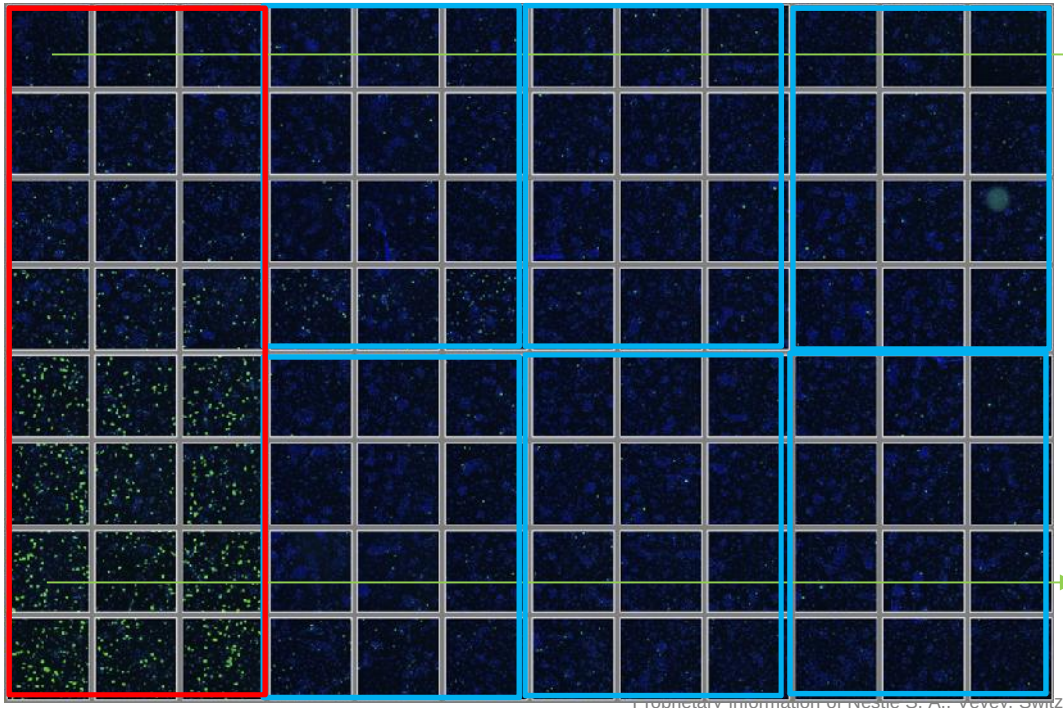
Genotoxicity γH2AX

HepaRG cells

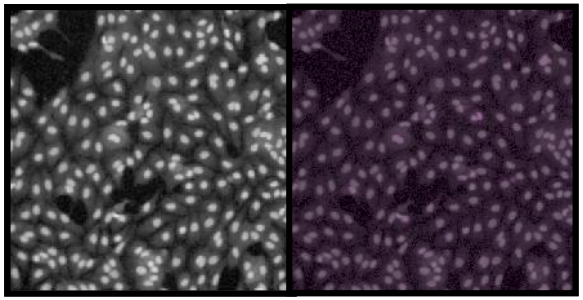




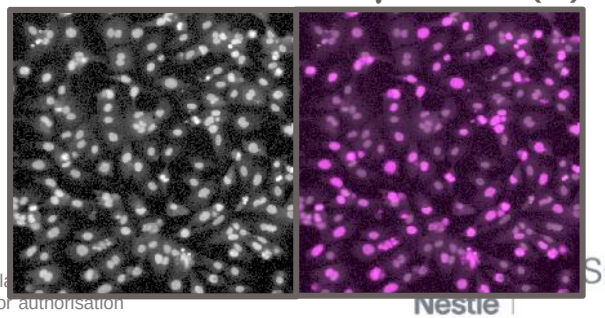




Cell count γH2AX (-)



Cell count γH2AX (+)

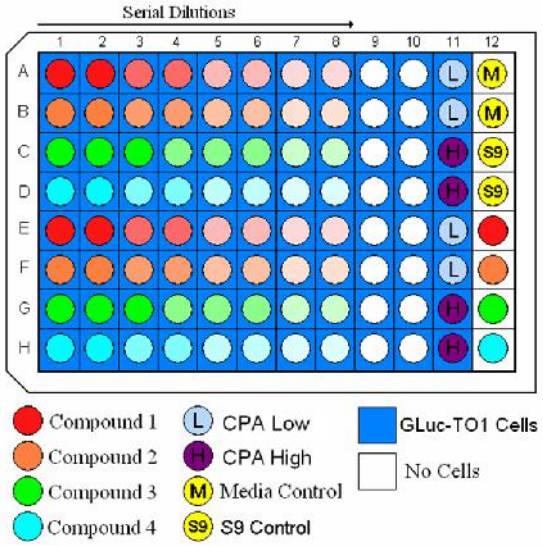


Gadd45α induction (Bluescreen)

gentronix
innovation and service

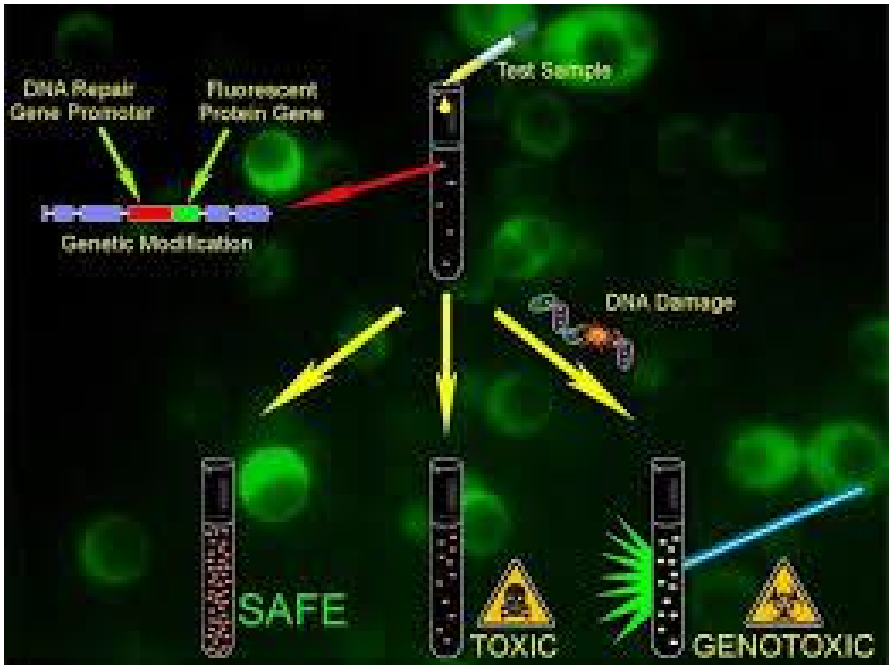
GlucT01 cells

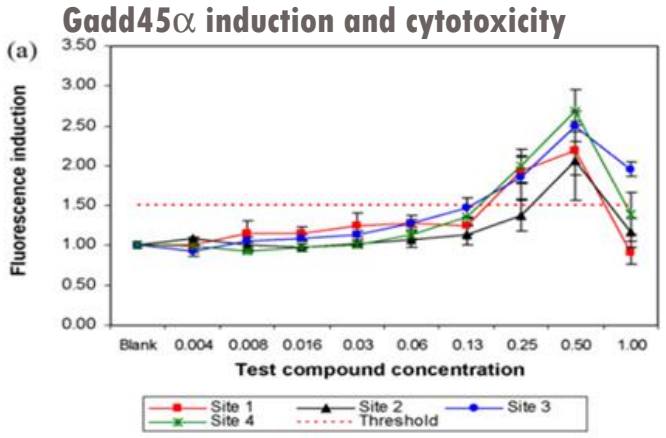
(S9/-S9)
(3h/24)



24h/recovery medium

Plate reader
RFU/RLU)





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Micronucleus formation

Thermo SCIENTIFIC

CHO cells
(3000 cell/well)

24h/37°C

CONTROLS
Mitomycin C
Etoposide

SAMPLES

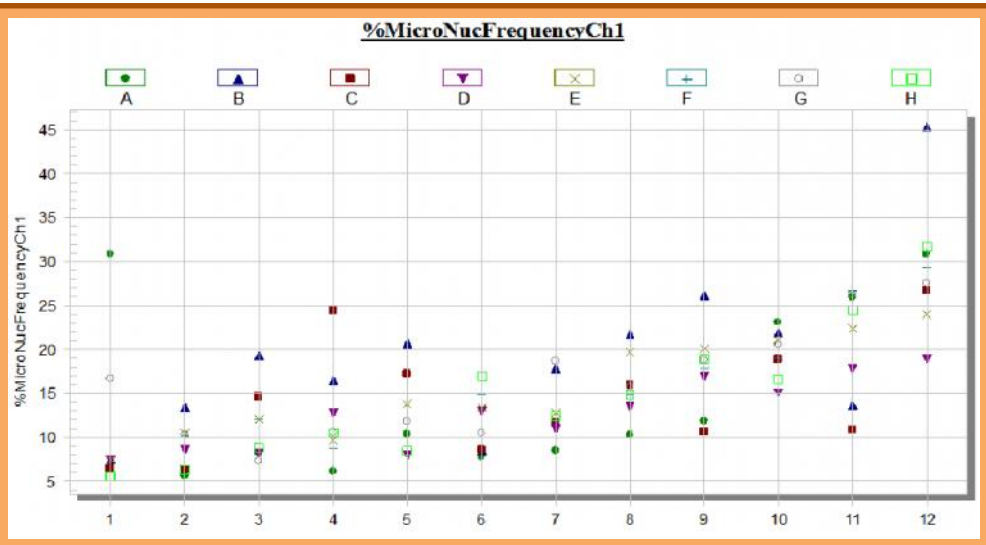
3h/24h/37°C

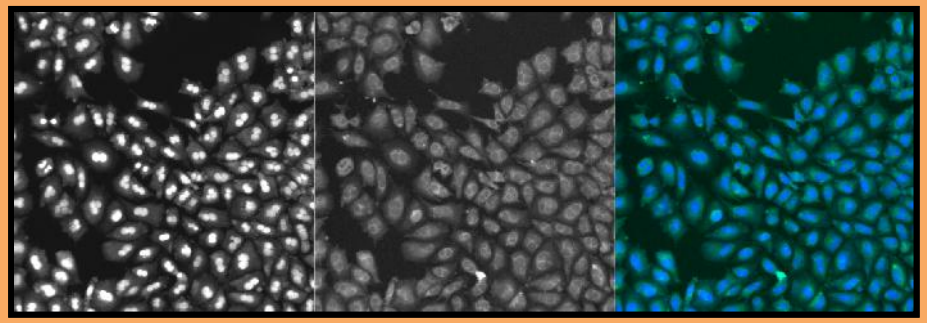
Cytokenesis block
Cytochalasin B

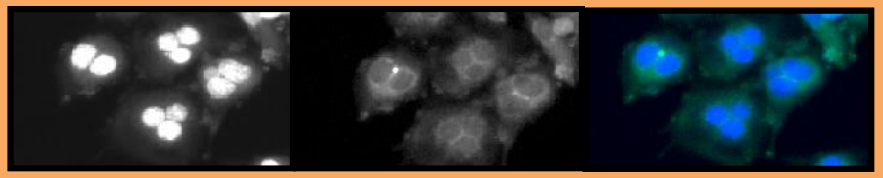
28h/37°C

Cell dye/Fixation

ToxInsight plate reader







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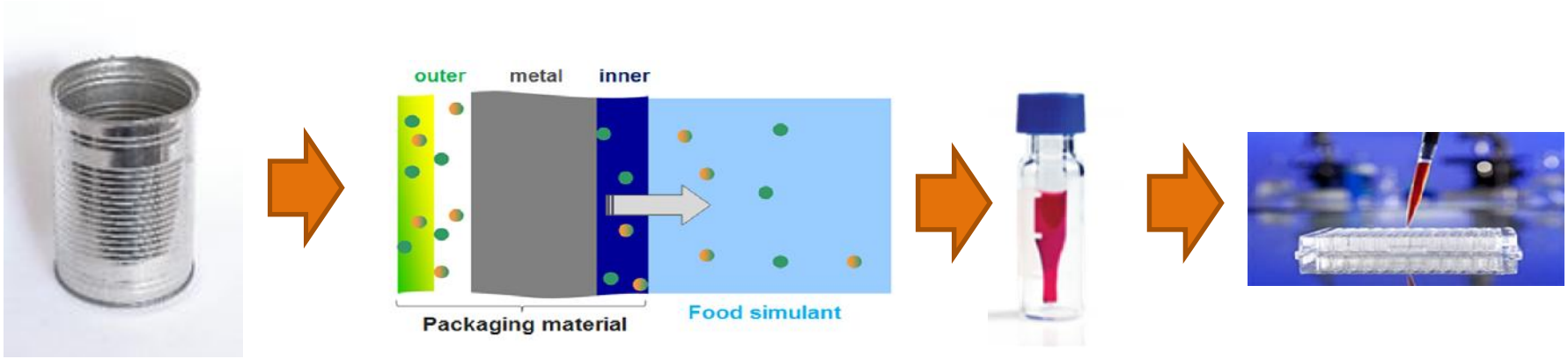
Approach application



Safety by design: bioassay data on experimental materials.

EthOH, 95%

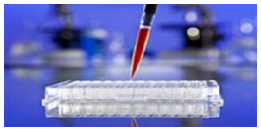
migration

10 days at 60°C

concentration

DMSO

Bioassays



Biological activity	Coating 1	Coating 2	Coating 3	Coating 4	Coating 5
Anti-estrogenic (ERα)					
PPARγ					
Anti-androgenic					
AhR					
Gadd45α					
Cytotoxicity					
AMES	*		N/A	N/A	N/A

?

Biological activity +

-

*AMES performed on similar coating

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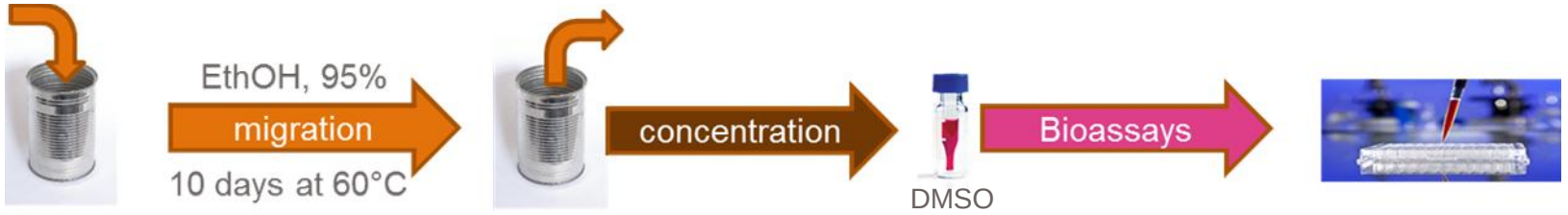
Biological activity in FCM-migrates?



Gaps and limitations need to be adressed:

- Relevance of migration studies (stability of the materials?)
- Identify causative agent(s)
  - Current analytical data did not reveal chemicals with alert for genotox (DNA-reactivity)
- Address mechanisms of genotoxicity
  - Mutagenic
  - No positive samples in Ames (no DNA reactive? Threshold?)
- …..

Refinement of bioassays intended to be applied for risk assessment?

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Bioassays can be negatively percieved in the packaging field, *some comments*:

«Poor quality/validation»:

- Data: not reliable, inconsistent, not repeatable within and across labs.
- Different assays for the same endpoints give different results

«Technical limitations»:

- Cells are not metabolically competent
- Packaging migrates cannot be tested *in vitro* because they produce unspecific cytoxicity

«Data cannot be interpreted»:

- Bioassays are too sensitive (everything lighting, many false positives)
- Bioassays are too insensitive (genotoxicity)
- They do not reflect *in vivo* situation
- We do not know how many need to be used
- They are not suitable for risk assessment
- ……

Refinement and reliability of bioassays intended to be applied for risk assessment???

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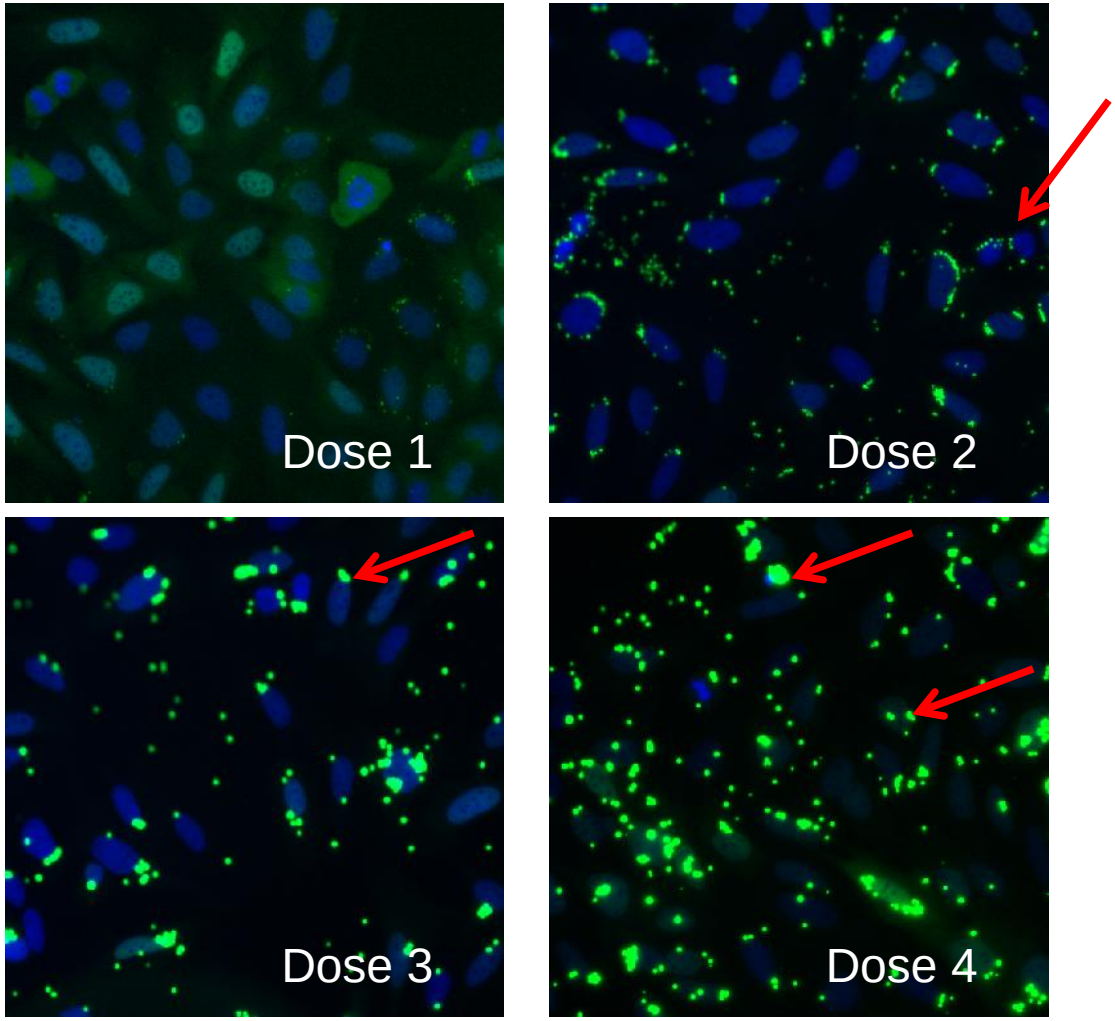
CROSSREACTION: TECHNICAL ARTIFACT (NOT BIOLOGICAL): PRECIPITATION RX?



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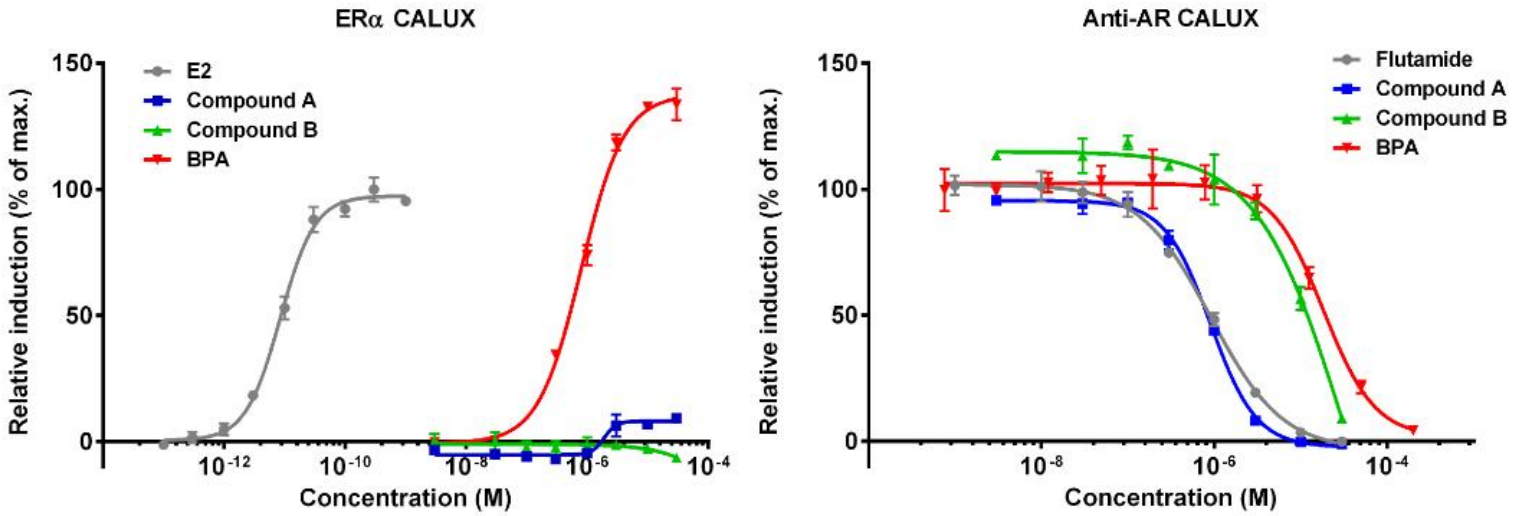
Safety by design:
evaluate raw materials early (e.g. monomers)



Compound A:

- PPARγ antagonist effect
- No antagonistic effect on ERα
- No agonistic effect on AR

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Monitoring cell viability in the same well: *Anti-AR activity as a relevant example*


Toxicology in Vitro xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

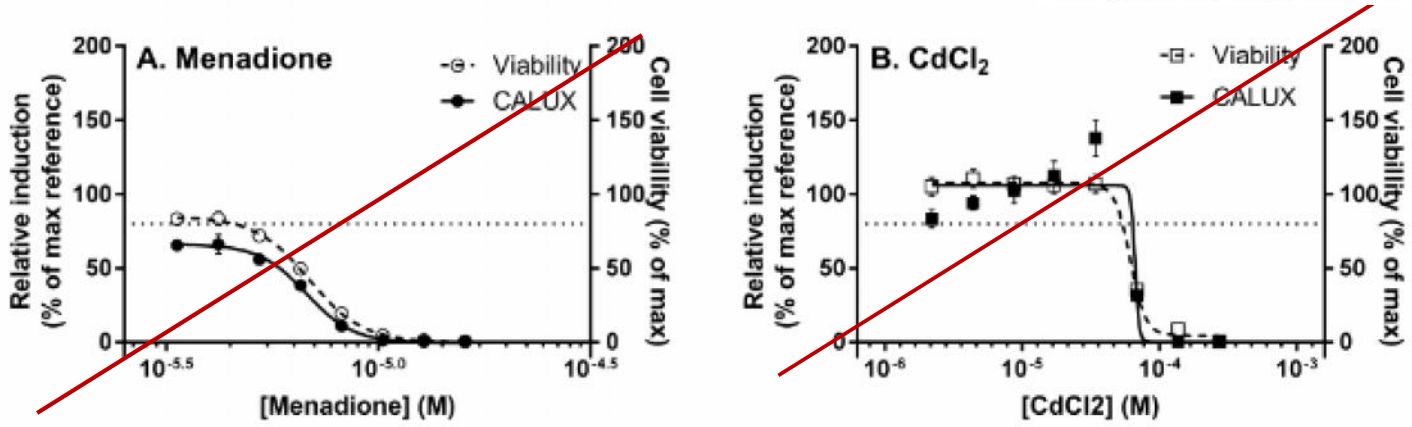
Toxicology in Vitro

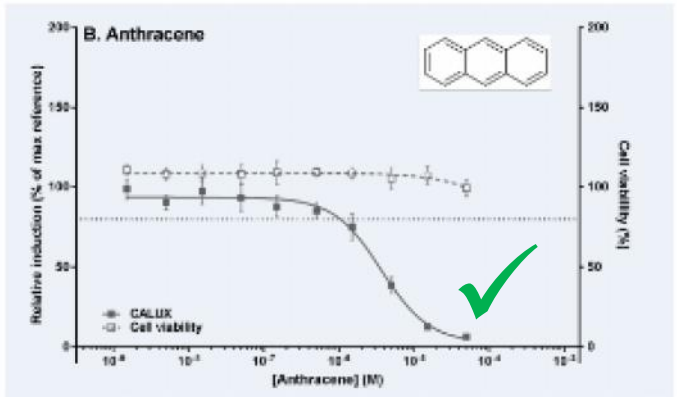
journal homepage: www.elsevier.com

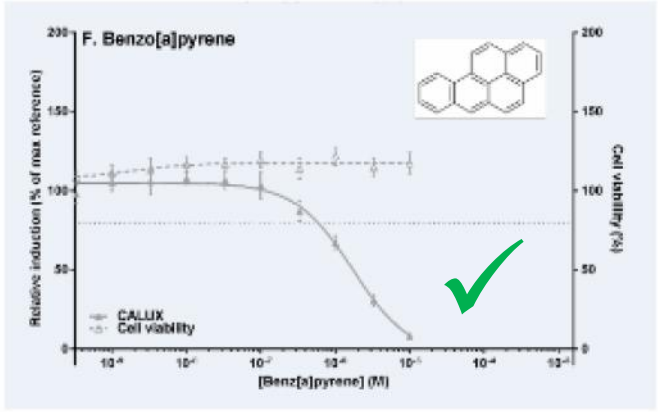
Differentiating true androgen receptor inhibition from cytotoxicity-mediated reduction of reporter gene transactivation *in vitro*

Maricel Marin-Kuan*, Karma C. Fussell, Nicolas Riederer, Helia Latado, Patrick Serrant, Julie Mollergues, Myriam Coulet, Benoit Schilter

Chemical Food Safety, Nestlé Research Centre, P.O. Box 44, CH-1000 Lausanne 26, Switzerland







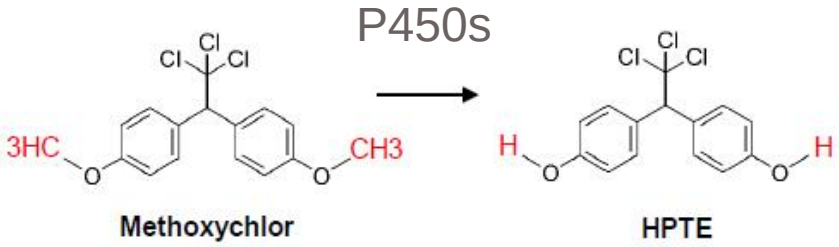
POSTER exhibited

**RealTime-Glo MT Cell Viability assay (Promega)*

Nestlé Research

Metabolic activity can be incorporated (+S9 liver fraction):
ERα-CALUX assay as an example

ALTEX Online first
published December 22, 2016
https://doi.org/10.14573/altex.1611021

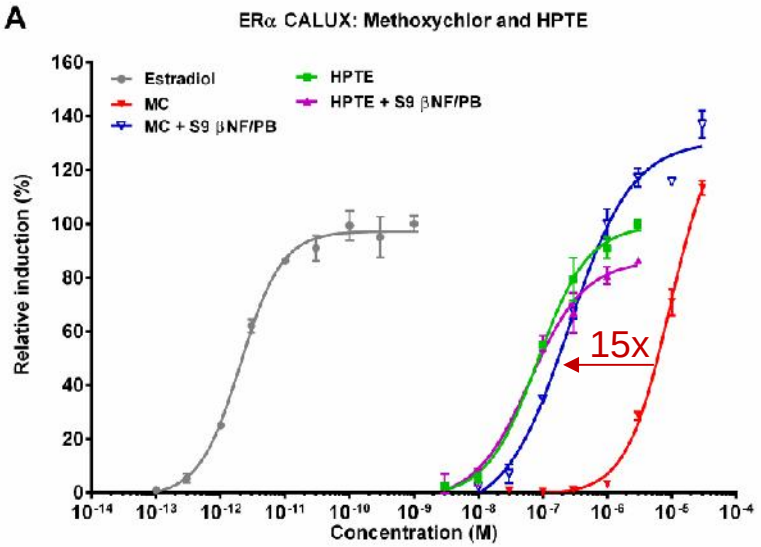


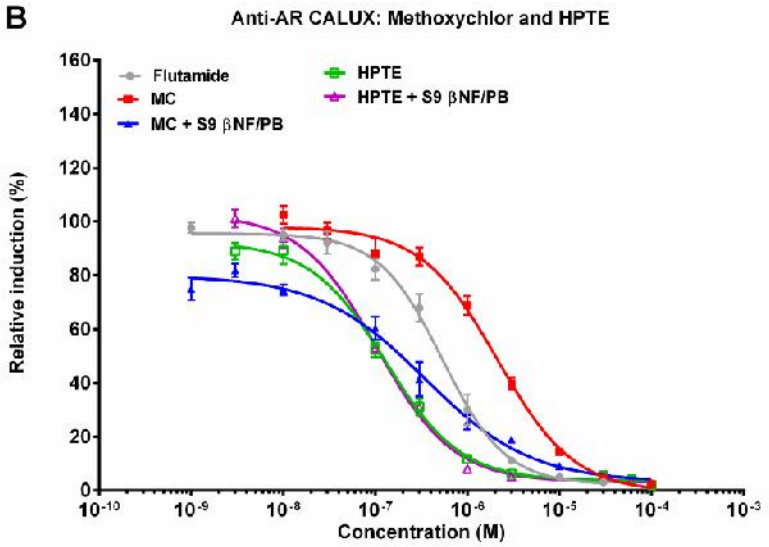
Research Article

Incorporation of a metabolizing system in biodetection assays for endocrine active substances

Julie Mollergues[1], Barbara van Vugt-Lussenburg[2], Christian Kirchnawy[3], Reka Anna Bondi[3], Rosan B. van der Lee[2], Maricel Marin-Kuan[1], Benoit Schilter[1] and Karma C. Fussell[1]
[1]Chemical Food Safety, Nestlé Research Centre, Lausanne, Switzerland; [2]BioDetection Systems, Amsterdam, Netherlands; [3]OFI - Austrian Research Institute for Chemistry and Technology, Vienna, Austria

(2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane)





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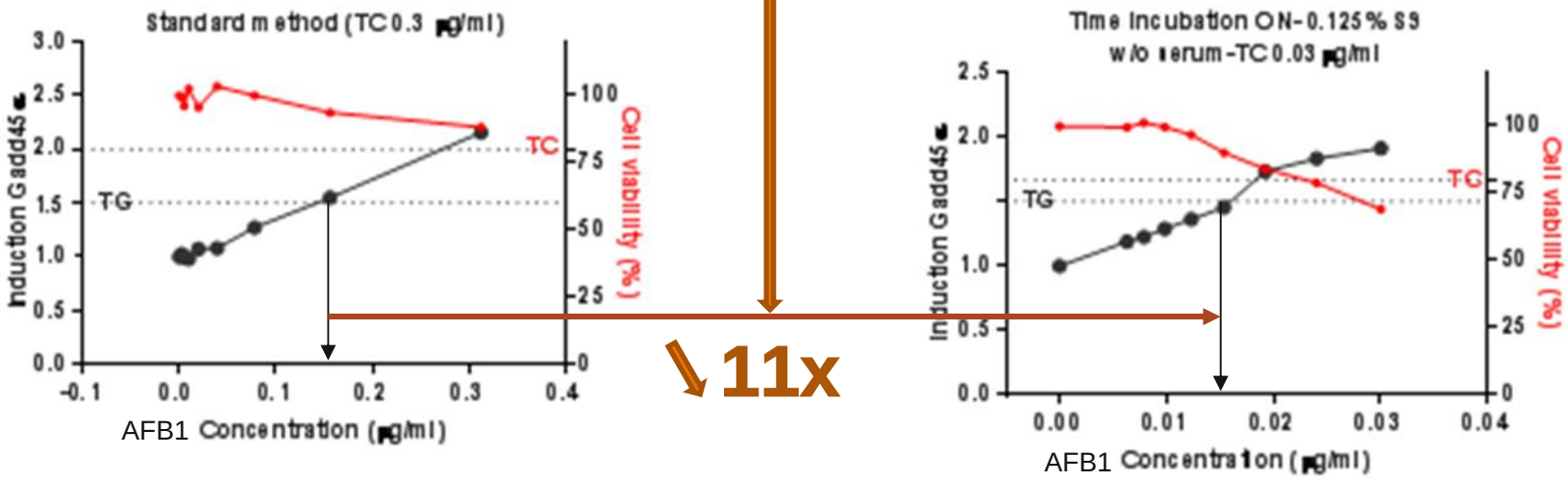
Sensitivity of genotoxicity tests can be improved

Marin Kuan M. et al in preparation

- Incubation time
- Activating system (+S9)
- Protein in the medium



- 3h
- 1% S9
- with serum

- O/N
- 0.125% S9
- w/o serum

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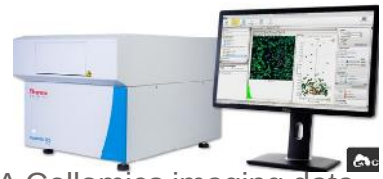
Bioassays: roles in packaging safety?

- To apply Cramer class III-TTC to structurally uncharacterized chemicals:
  - Exclude chemicals of the «cohort of concern»
    - Genotox, AhR,
  - To exclude chemicals with genotoxic alert
  - To exclude AChE inhibitors
- Identify chemicals associated with some endocrine activity:
  - To be managed early
- Identify presence of unknown chemicals of high toxic potency
- Identify potential for mixture effects

ILSI Europe
International Life Sciences Institute

Refinement of *in vitro* exposure and method performance to ensure reliability and trust of bioassays application for risk assessment needs attention

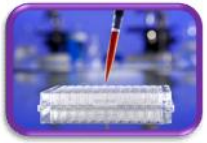
Fondation of efficient food safety assessment



HCA Cellomics imaging data

Hamilton Starline data

multidetection microplate reader



data management system (ELN and spotfire)

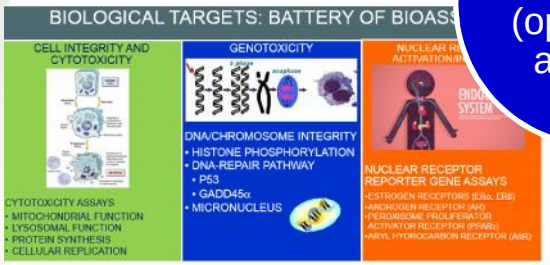
Throughput & efficiency (automation)

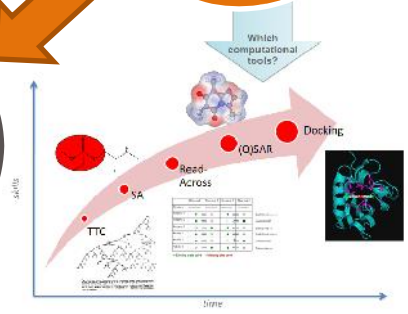
In vitro toxicology (optimized assays)

Food safety

In silico

Risk assessment





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