

# French research project on oligoesters migrating from food can coatings (OLIGO)

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# Outline

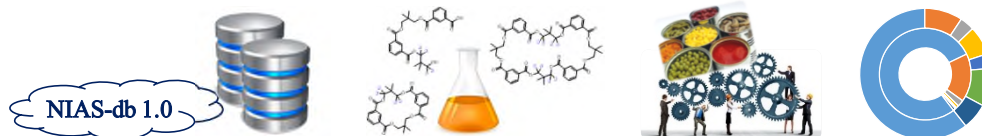
- Introduction to  LABERCA



- Context and research question



- A preliminary study on vegetable cans



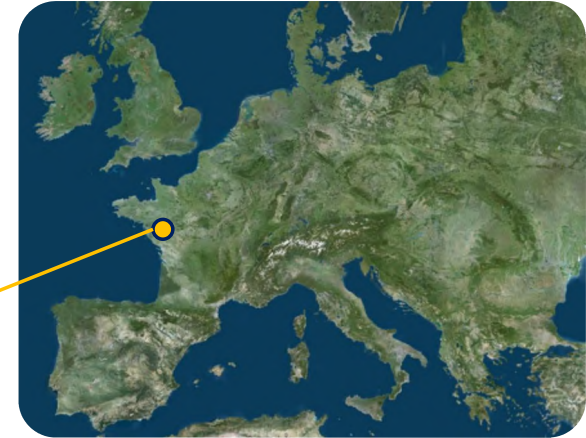
- OLIGO, an on-going collaborative project



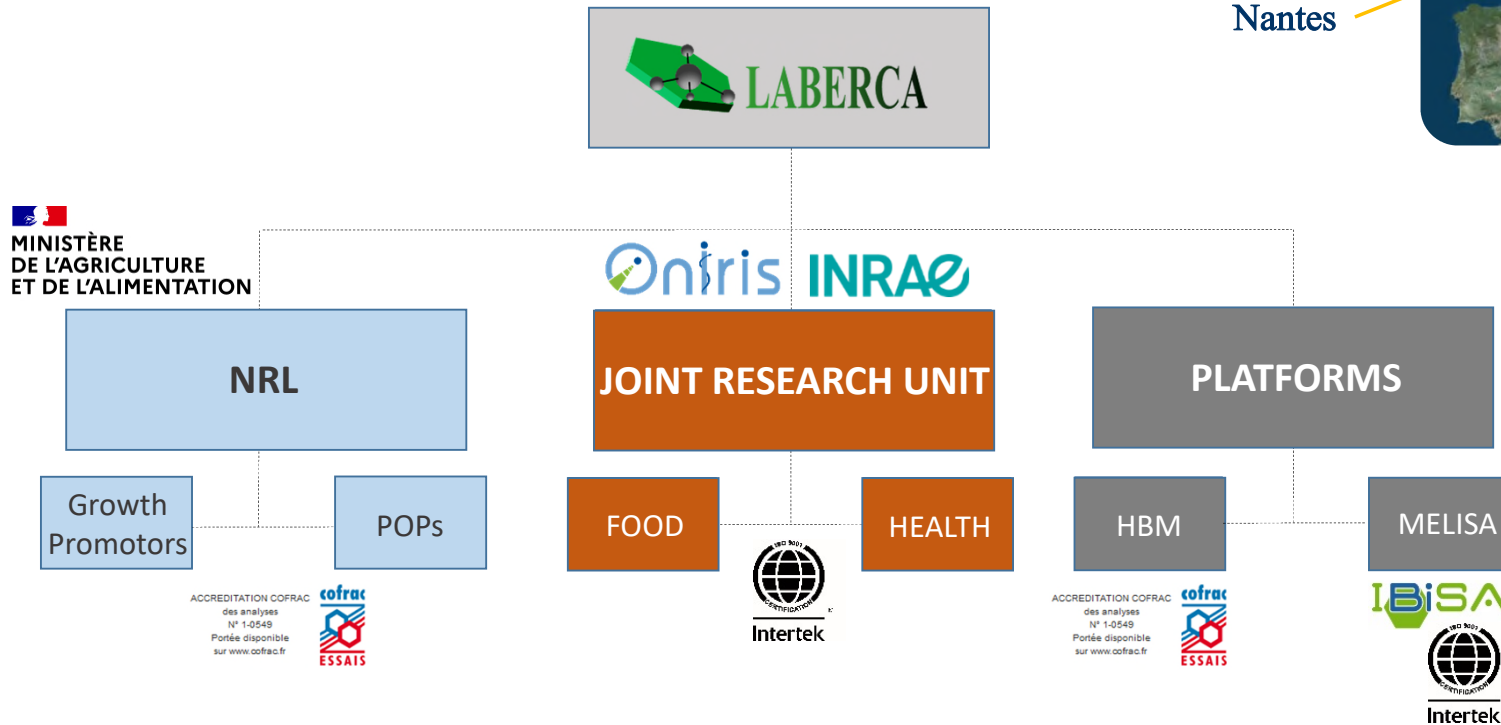
# LABERCA

## LABoratoire d'Étude des Résidus et Contaminants dans les Aliments

~70 persons

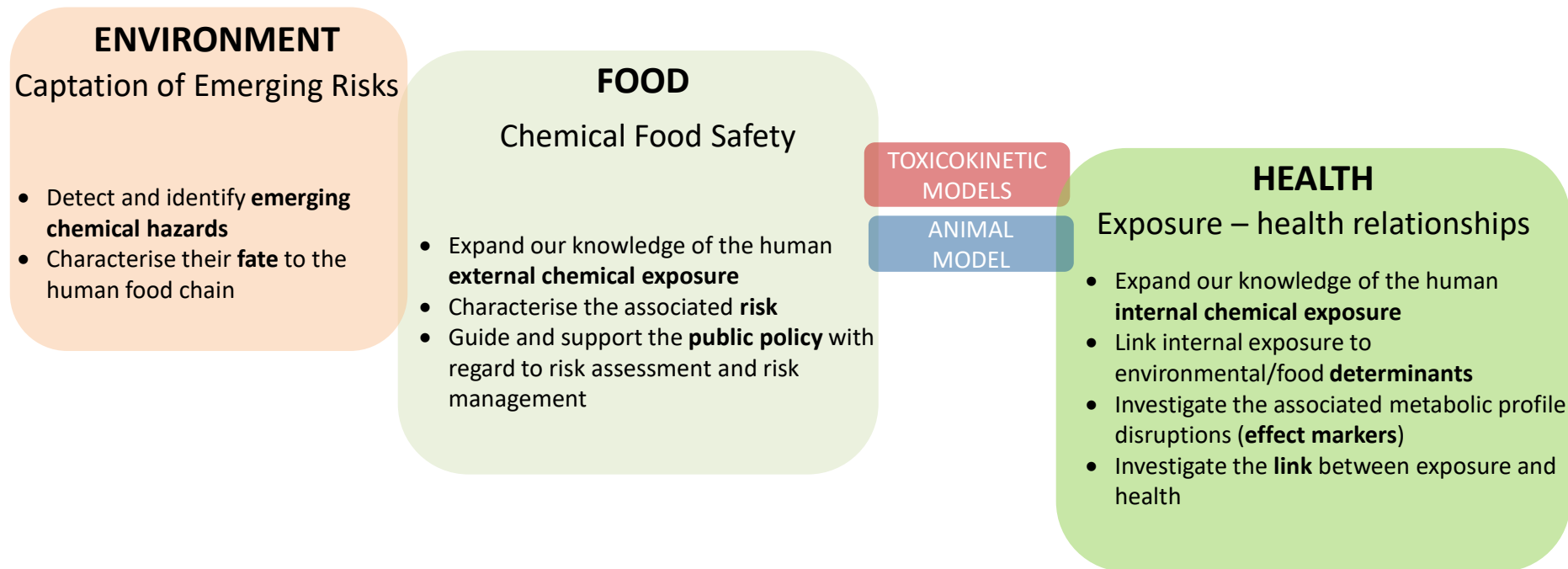


Nantes



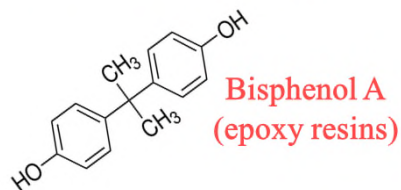
## Research question

Expanding knowledge of the human chemical exposome to better investigate possible associations between exposure and human health





## FCM / varnishes and coatings



SML 0.05 mg/kg food  
Not permitted for infant food



Banned since 2014  
⇒ Distortion of the Single market



On-going re-evaluation  
⇒ Expected decrease of TDI

### Questions

❓ Which marketed alternatives?

✅ Starting substances evaluated

❓ Non-intentionally added substances (NIAS)

❓ *Predictable*

❓ *Non predictable*

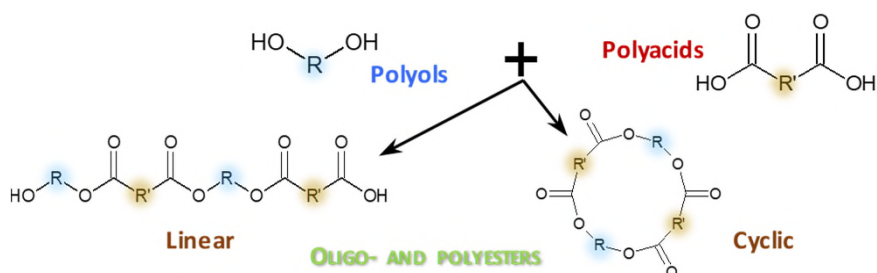
Science front



# Preliminary study

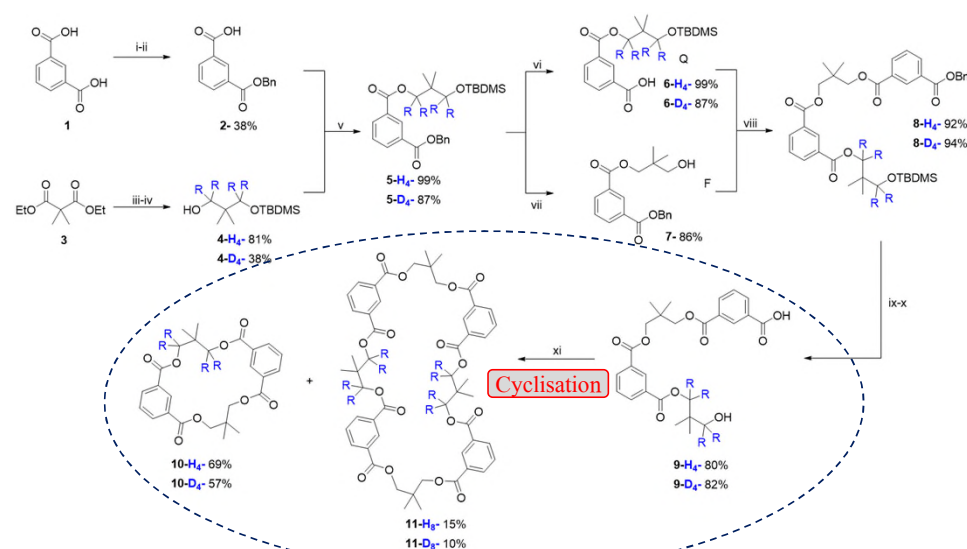
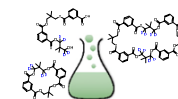
# Synthesis of reference compounds

## *A priori* indication of polyesters



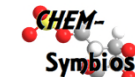
## Lack of reference oligoesters

$\Rightarrow$  Synthesis of native and D-labelled combinations of neopentyl glycol (NPG) & isophthalic acid (iPA)



### Chemical purity

- NMR >95%
- LC-ESI(+)-HRMS no impurities





## Combinations of monomers (ResAP(2004)1, + nadic acid)

### 17 polyols

Abrev.	Name(s) Isomer	Number of functions	Monoisotopic mass (Da)
EG	Ethylene glycol	2	62
PD	Propanediol 1,2-Propanediol; Propylene glycol 1,3-Propanediol	2	76
BD	Butanediol 1,4-Butanediol 1,3-Butanediol	2	90

### 15 polyacids

Abrev.	Name(s) Isomer	Number of functions	Monoisotopic mass (Da)
G3	Glycerol		
NPG	Neopentyl OA Oxalic acid	2	90
DEG	Diethylene MA Maleic/fumaric acid	2	116
DHB	Dihydroxy Maleic acid 1,2-D Fumaric acid 1,3-D SuA Succinic acid 1,4-D IA Itaconic acid	2	118
HD	1,6-Hexane AA Adipic acid	2	146
PG2	Dipropyl PA Phthalic acid	2	166
TMP	1,1,1-Trim o-Phthalic acid (oPA)		
CHDM	1,4-Bis(hyc Isophthalic acid (iPA)		
TMPD	2,2,4-Trim Terephthalic acid (tPA)		
TEG	Triethylen THA Tetrahydrophthalic acid	2	170
PG3	Tripropyle 3,4,5,6-Tetrahydrophthalic acid <sup>f</sup>		
EG4	Tetraethyl 1,2,3,6-Tetrahydrophthalic acid <sup>f</sup>		
TCDDM	Tricyclo HHA Hexahydrophthalic acid	2	172
BHCP	2,2-Bis(4-h Hexahydroterephthalic acid Hexahydroorthophthalic acid <sup>f</sup>		
	NA Nadic acid	2	182
	AZA Azelaic acid	2	188
	SeA Sebacic acid	2	202
	TMA Trimellitic acid	3	210
	DDDA n-Dodecanedioic acid	2	230
	SIA 5-Sulphoisophthalic acid	2	246
	PMA Pyromellitic acid	4	254

R-script  
Linear, partially/fully cyclised → ~76×106 combinations  
< 1000 Da 2–18 monomer units

## NIAS-db: an open-source software

- Chromium web browser
- *Shiny* framework

Freely available  
doi [10.15454/HHY2Z2](https://doi.org/10.15454/HHY2Z2)



## 12 cans

Supermarket  
Vegetables  
3PC, 2PC



## Coatings

n=40  
Scrapped  
ACN extraction



## Data acquisition

Non-targeted analysis



*LC/ESI(+)-HRMS*

Omer *et al.* (2018, 2019)

## Data mining

Automated peak picking  
Manual curation



## Identification

Schymanski scale

## Identification

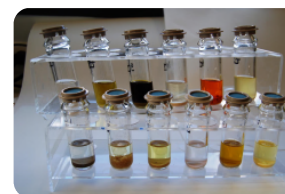
List of  
compounds  
present in  
compounds

## Foodstuffs

Juice  
Drained



## Sample preparation



## Data acquisition

## Data treatment

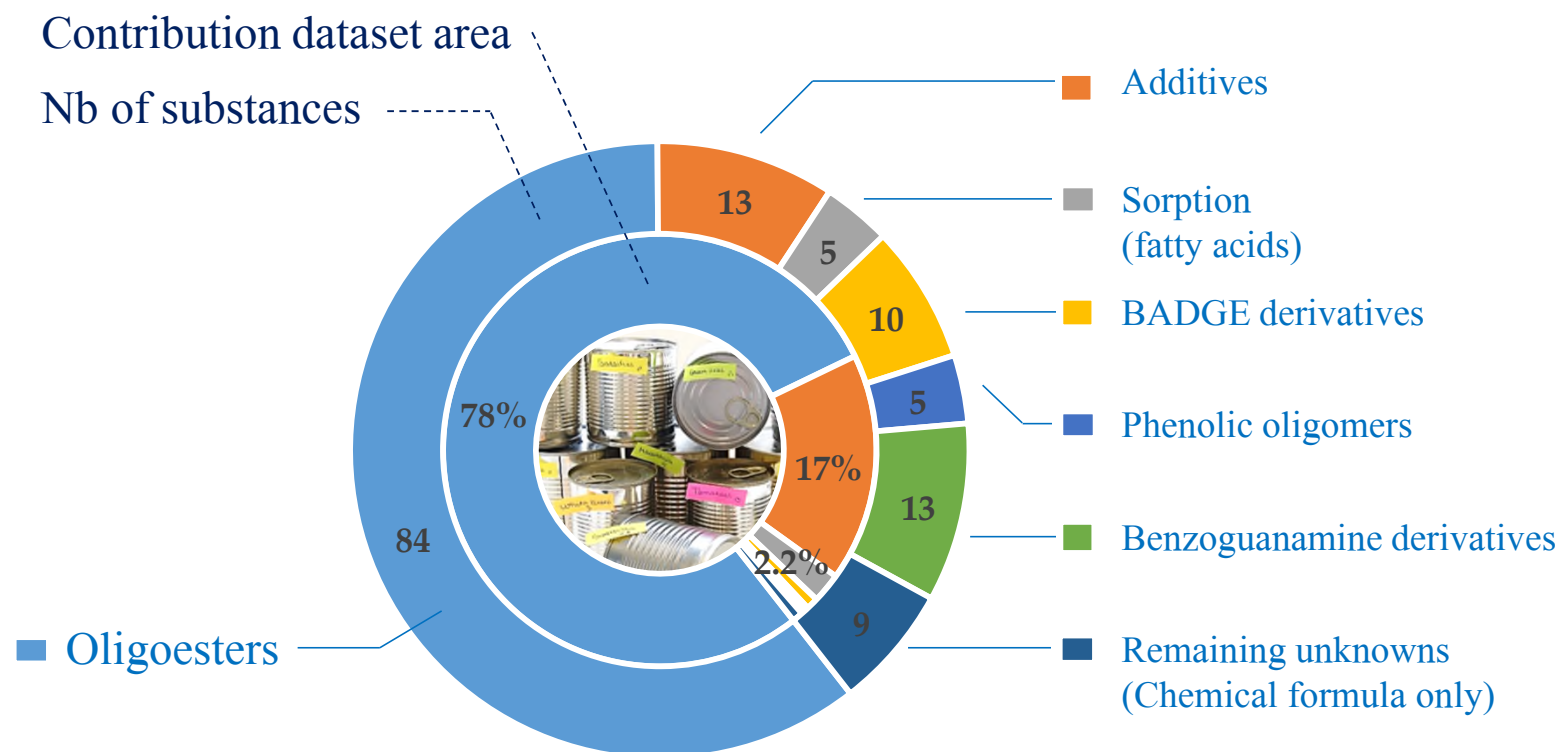
Suspect screening  
Manual curation

## (semi-)Quantification





139 substances in coatings



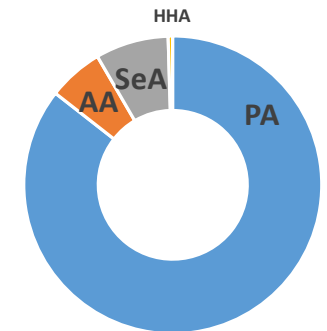
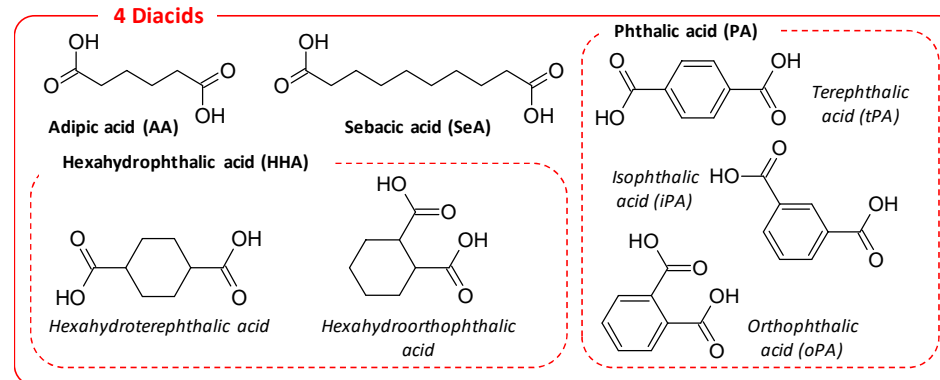
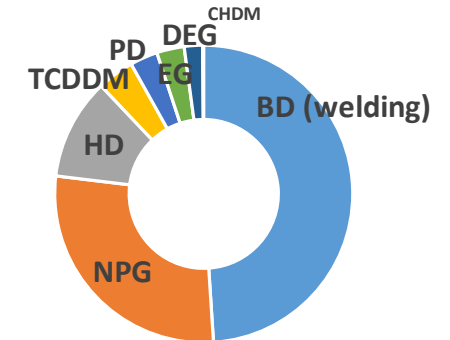
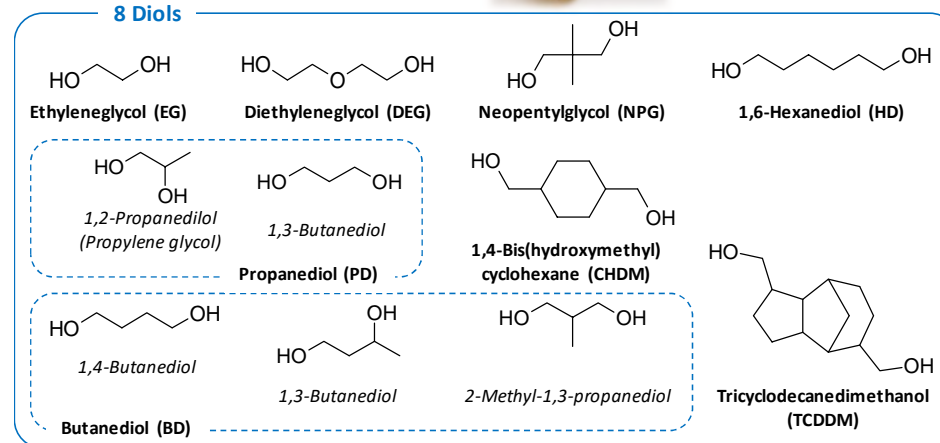
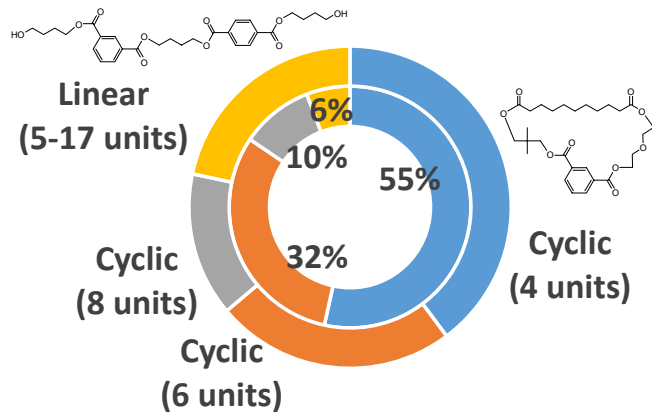
# Preliminary study

# Oligoesters in coatings



## Oligoesters in coatings

84 combinations  
78% relevant signal  
38 out of 40 coatings  
Most are Cramer class III



# Preliminary study



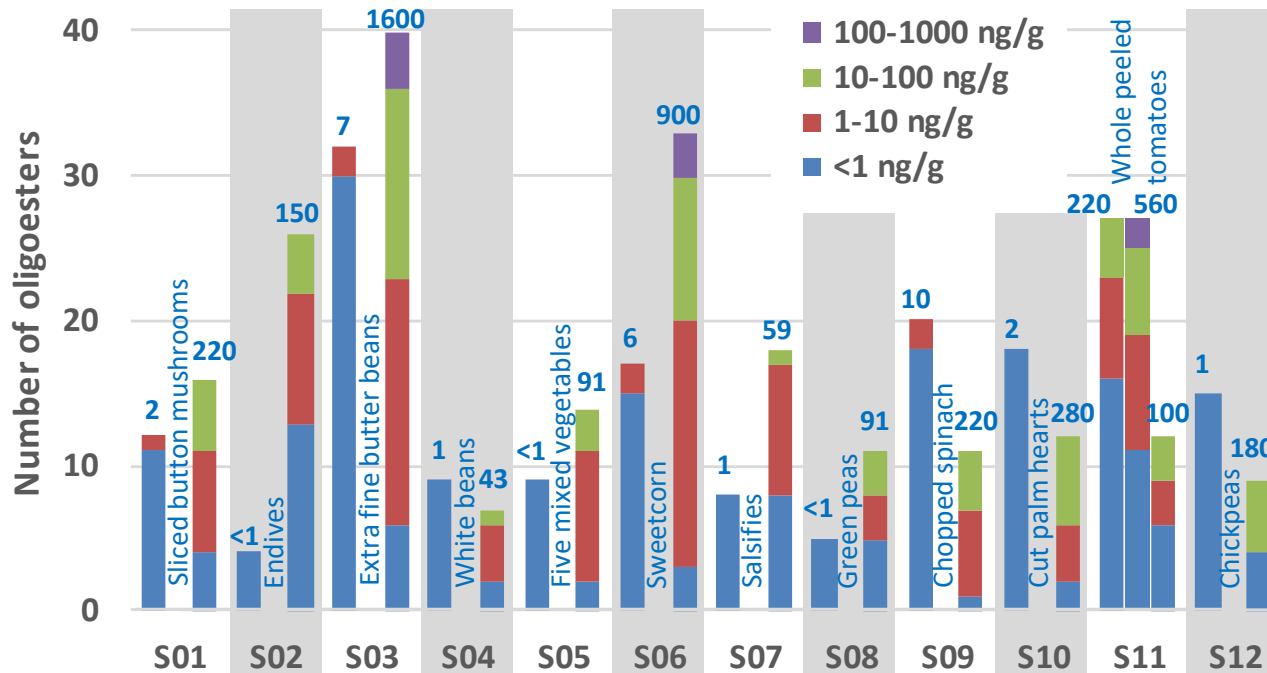
# Oligoesters in foodstuffs

n=68 combinations migrating to drained vegetables

Most prevalent combinations  
(n=18, mean >5 µg/kg)

## Σoligoesters

330 µg/kg on average (43–1600 µg/kg)



- c(2NPG+2PA)
- c(2DEG+2PA)
- c(DEG+NPG+PA+SeA)
- c(EG+NPG+PA+SeA)
- c(DEG+NPG+2PA)
- c(EG+DEG+2PA)
- c(EG+DEG+PA+SeA)
- c(EG+NPG+PA+AA)
- c(2BD+PA+AA)
- c(2NPG+PA+AA)
- lin(3NPG+PA+AA)
- c(2NPG+2AA)
- lin(3NPG+2PA)
- c(NPG+HD+2PA)
- c(2HD+2PA)
- c(2BD+2PA)
- lin(3DB+2PA)
- c(2BD+PA+SeA)



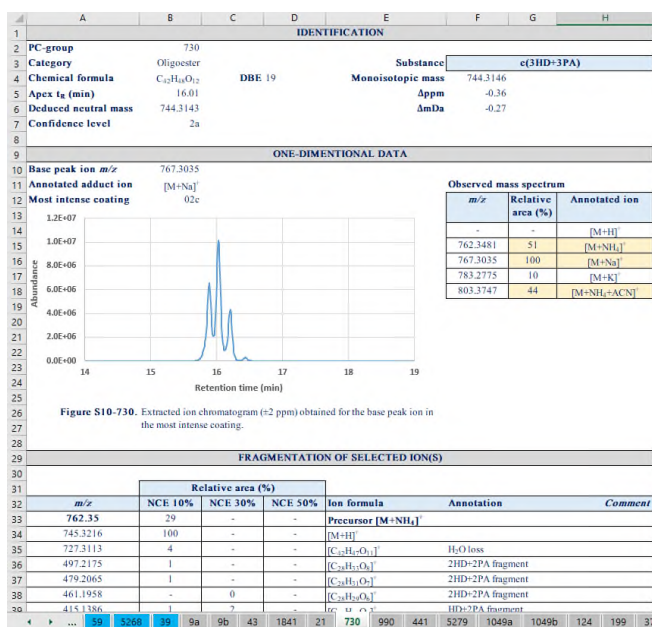
More details in [Research Paper](#)

Thorough investigation of non-volatile substances extractible from inner coatings of metallic cans and their occurrence in the canned vegetables

Ronan Cariou<sup>a,\*</sup>, Matthieu Rivière<sup>b</sup>, Sébastien Hutinet<sup>a</sup>, Asmaa Tebbaa<sup>a,b</sup>, Didier Dubreuil<sup>b</sup>, Monique Mathé-Allainmat<sup>b</sup>, Jacques Lebreton<sup>b</sup>, Bruno Le Bizec<sup>a</sup>, Arnaud Tessier<sup>b</sup>, Gaud Dervilly<sup>a</sup>



doi [10.1016/j.jhazmat.2022.129026](https://doi.org/10.1016/j.jhazmat.2022.129026)



## Detailed supporting information

- NIAS-db
- Synthesis
- Sample preparation development
- 1 Excel sheet per compound (EIC, main ions, annotated MS<sup>2</sup> fragments)

## Funding and support



- 2021 ANR generic call  
Food and Food Systems committee  
⇒ OLIGO ⇒ 469 k€, 2021-2025  
(ANR-21-CE21-0005)



- Competitiveness cluster (no funding)  
Agri-food area, North-west France





## Partners



Ronan Cariou  
P1-LABERCA



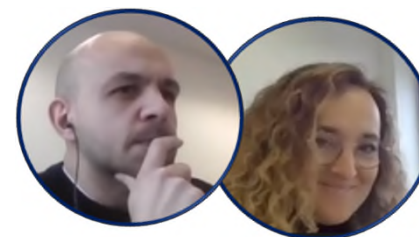
Arnaud Tessier  
P2-CEISAM



Marie-Christine Chagnon  
Isabelle Séverin  
P3-LNC



Nicolas Cabaton  
P4-TOXALIM



Bruno Teste  
Nawel Bemrah  
P5-Anses



Grégory Pieters  
CEA



## SAB members



Cristina Nerín  
University of Zaragoza, Spain



Thomas Simat  
Technische Universität Dresden, Germany

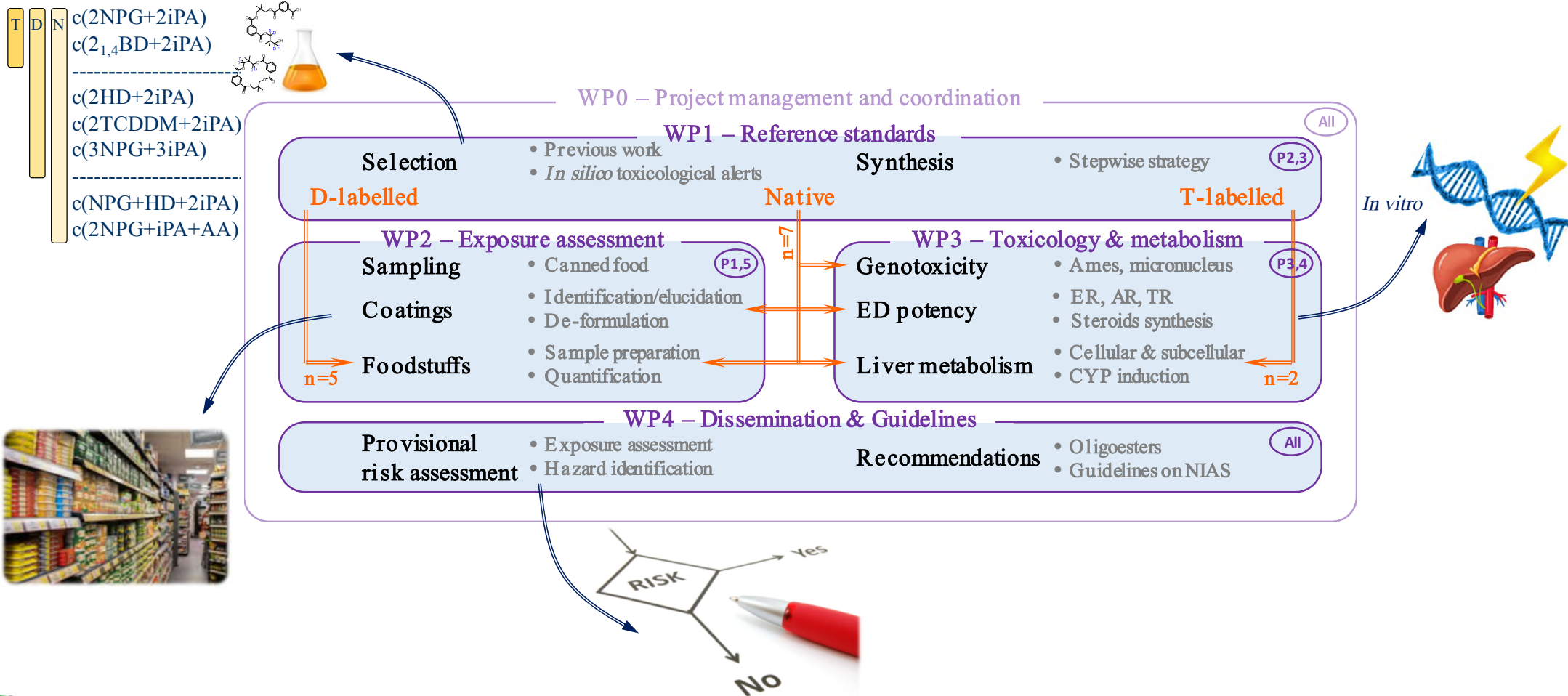


Åke Bergman  
Örebro University, Sweden



Eric Barthélémy  
EFSA, Italy

## Oligoesters migrating from food can coatings – Filling risk assessment gaps







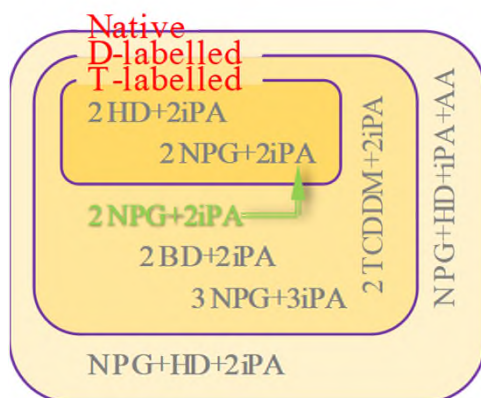
Thank you for your attention!



# Annexes, just in case...

Work package number	WP1	Schedule	M1 to M24
Work package title	<b>ORGANIC SYNTHESIS OF REFERENCE AND LABELLED STANDARDS</b>		
Participants	P2-CEISAM (lead)	P3-LNC	P1-LABERCA
Objectives	<ul style="list-style-type: none"> <li>To predict the toxicity of oligoesters with <i>in silico</i> methodologies</li> <li>Availability of a selection of representative oligoesters to all consortium partners</li> <li>Full characterisation of products by NMR and MS techniques, with high degrees of purity</li> </ul>		
Deliverables	<ul style="list-style-type: none"> <li>D1.1 Report justifying the finalised list of oligoesters to be synthesised M3</li> <li>D1.2 Availability of 7 native cyclic oligoesters, fully characterised M12</li> <li>D1.3 Availability of 2 tritium-labelled cyclic oligoesters, fully characterised M18</li> <li>D1.4 Availability of 5 deuterium-labelled cyclic oligoesters, fully characterised M24</li> </ul>		

## Task 1.1: Determination of monomer combinations to be synthesised

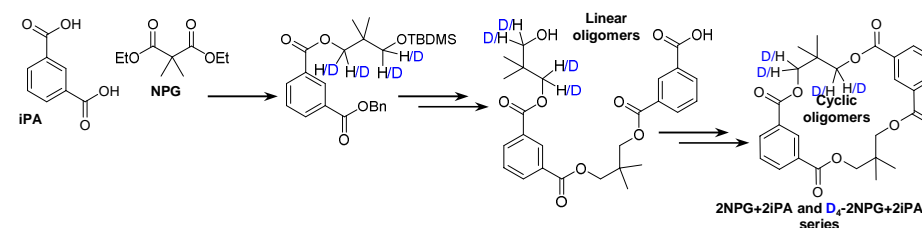


## Task 1.2: Synthesis of 7 native cyclic oligoesters

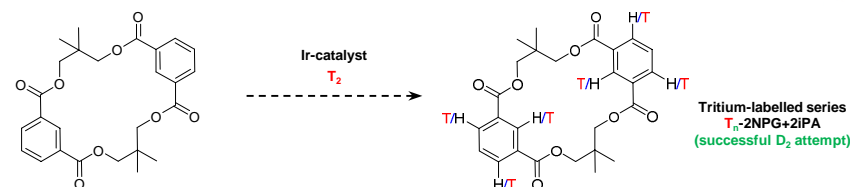
n=7, chemical purity >97%  
~35 new intermediate products

## Task 1.4: Synthesis of 5 deuterium-labelled cyclic oligoesters

n=5, chemical purity >97%



## Task 1.3: Synthesis of 2 tritium-labelled cyclic oligoesters





Work package number	WP2	Schedule	M13 to M36
Work package title	EXPOSURE ASSESSMENT		
Participants	P1-LABERCA (lead)	P5-ANSES	
Objectives	<ul style="list-style-type: none"> <li>To document the prevalence of oligoesters in metal can and lid coatings marketed for France</li> <li>To document the occurrence of oligoesters in the diet in France</li> </ul>		
Deliverables			Due date
D2.1	Stored sample set of representative coatings and foodstuffs		M18
D2.2	Database on the occurrence of oligoesters in can coatings marketed for France		M30
D2.3	Characterised method dedicated to oligoesters in foodstuffs		M30
D2.4	Report on the occurrence of oligoesters in foodstuffs in France		M36

## Task 2.1: Can sampling

- Representative sampling plan of food-containing metal FCMs (n=100 items, ~350 coatings)

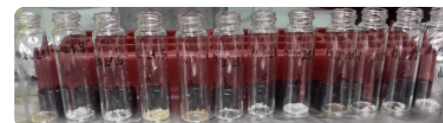


- Based on food consumption habits of the French population
- Food type stratification, can shape/capacities, brand names and retailers will be considered
- Vegetables, seafood, meat products, ready-made dishes, beverages and infant milk formulas, ...
- No seasonal or regional specificities are anticipated



- Food separated from coatings

## Task 2.2: Identification of oligoesters in can coatings



- Acetonitrile extraction
- Non-targeted LC-ESI(+)-HRMS acquisition
- Data mining (R, databases, MS<sup>2</sup>, Schymanski scale)

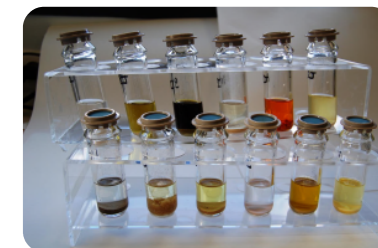


- Analysis of monomers after hydrolysis (n=20)



## Task 2.3: Sample preparation for determining oligoesters in foodstuffs

- Sample preparation (LLE, lipids removal)
- Characterisation of performances



## Task 2.4: Quantification of oligoesters in foodstuffs

- Semi-quantification
- Isotopic dilution (D-labelled standards)



# Work packages

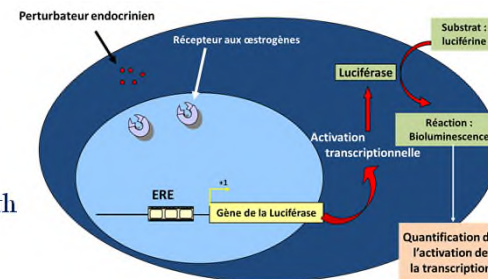
# WP3 – Toxicology and metabolism

Work package number	WP3	Schedule	M13 to M42
Work package title	TOXICOLOGY AND METABOLISM		
Participants	P3-LNC (co-lead)	P4-TOXALIM (co-lead)	P1-LABERCA
Objectives	<ul style="list-style-type: none"> <li>To assess the genotoxicity and ED potency of the selected oligoesters</li> <li>To elucidate the human liver metabolism of two representative oligoesters</li> <li>To assess the effects of oligoesters on key CYP450 enzymes</li> </ul>		
Deliverables			Due date
D3.1	Report on the genotoxicity and mode of action with the ED potency of major oligoesters and their main metabolites		M36
D3.2	Report on the human liver metabolism of 2 radiolabelled oligoesters		M36
D3.3	Report on the effects of oligoesters on key CYP450 enzymes		M36

## Task 3.2: Endocrine disruption potency

Level 2 of the OECD guidance

- (anti)Estrogenic activity (OECD guideline no. 455)  
->Transcriptional activation assay (TA) on human cell line (hERαHeLa-9903 cells)
- (anti)Androgenic activity  
->MDA-kb2 cell line stably transfected with the murine mammalian tumor virus (MMTV)-luciferase.neo reporter gene construct

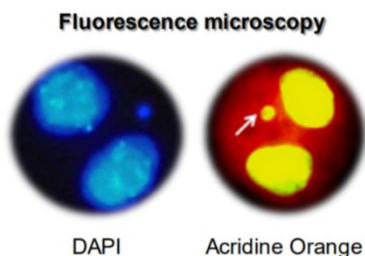


## Task 3.1: Genotoxicity assays

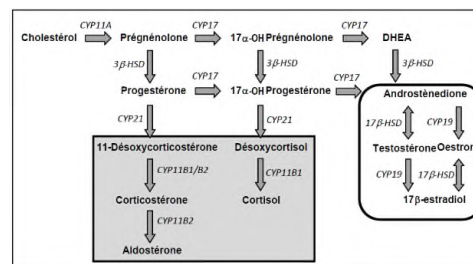
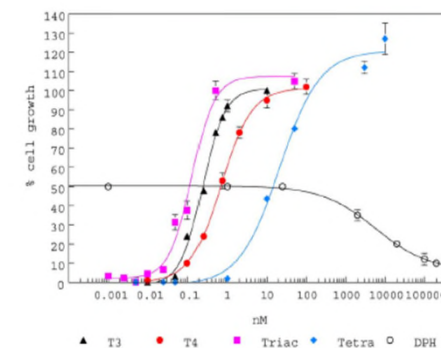
OECD Guidelines

### OECD GUIDELINE FOR THE TESTING OF CHEMICALS

- Bacterial reverse mutation assay on *Salmonella typhimurium* (Ames test)  
-> Detection of gene mutations (OECD guideline no. 471)
- Micronucleus assay  
-> Detection of chromosomal aberrations in human hepatoma cell line HepG2 (OECD guideline no. 487)



- Thyroid hormone receptors (TRs)  
->T-Screen bioassay, involving the GH3 rat tumor cell line that expresses the thyroid hormone receptors and depends on the presence of T3 hormone for growth



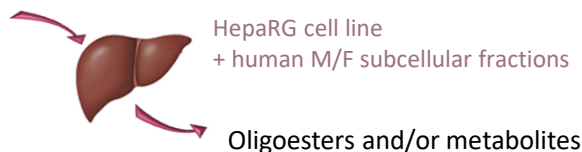
- EDCs that induce change in the production of estradiol and testosterone (indirect mechanism of action)  
-> H295R steroidogenesis assay (OECD guideline no. 456) involving a human adenocarcinoma cell line (NCI-H295R)

Work package number	WP3		Schedule	M13 to M42
Work package title	TOXICOLOGY AND METABOLISM			
Participants	P3-LNC (co-lead)	P4-TOXALIM (co-lead)	P1-LABERCA	
Objectives	<ul style="list-style-type: none"> <li>To assess the genotoxicity and ED potency of the selected oligoesters</li> <li>To elucidate the human liver metabolism of two representative oligoesters</li> <li>To assess the effects of oligoesters on key CYP450 enzymes</li> </ul>			
Deliverables				Due date
D3.1	Report on the genotoxicity and mode of action with the ED potency of major oligoesters and their main metabolites			M36
D3.2	Report on the human liver metabolism of 2 radiolabelled oligoesters			M36
D3.3	Report on the effects of oligoesters on key CYP450 enzymes			M36

## Task 3.3: Human liver metabolism

### 1) Biotransformation pathways

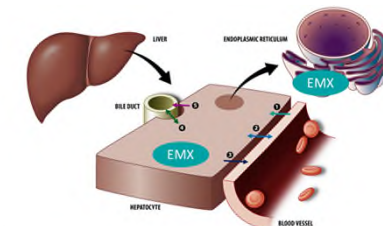
$2^3\text{H}$ -labelled oligoesters



- Mass balance, metabolic profiling (radio-HPLC detection)
- Structural characterization of main metabolites (LC-MS<sup>n</sup>, NMR)
- Production, purification and test of their ED potencies

### 2) Effects on key CYP450 enzymes

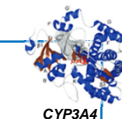
HepaRG cell line



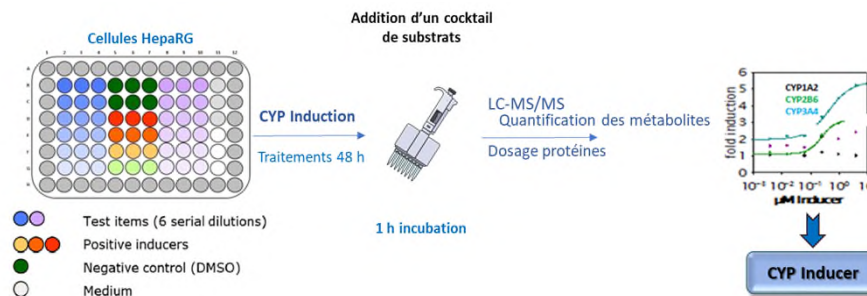
Localisation des EMX de phase I et II dans un hépatocyte (d'après van Groen BD et al., Pharmacol Rev., 2021) 1-5. Transporteurs hépatiques

Cytotoxicity assays: range of non-cytotoxic concentrations for the 7 oligoesters

Key CYP450 enzymes' induction assay for the 7 oligoesters (according to draft test guideline TM2009-14)



→ Their potential to induce CYP1A2, CYP3A4 and CYP2B6 activities (mediated *via* AhR, PXR and CAR receptors respectively) will be measured.



Bernasconi et al., *Toxicol In Vitro*, 2019  
<https://doi.org/10.1016/j.tiv.2019.05.019>

<b>Work package number</b>	WP4		<b>Schedule</b>	M30 to M42	
<b>Work package title</b>	<b>DISSEMINATION AND GUIDELINES</b>				
<b>Participants</b>	P1-LABERCA (lead)	P2-CEISAM	P3-LNC	P4-TOXALIM	P5-ANSES
<b>Objectives</b>	<ul style="list-style-type: none"> <li>To valorise produced original data</li> <li>To perform a provisional risk assessment</li> <li>To draft official guidelines for the risk assessment of NIAS in coatings</li> </ul>				
<b>Deliverables</b>	<ul style="list-style-type: none"> <li>D4.1 Submitted peer-review articles on genotoxicity/ED potency, metabolism and prevalence in foodstuffs</li> <li>D4.2 Submitted peer-review article on provisional risk assessment of oligoesters</li> <li>D4.3 Drafted ANSES guideline on the risk assessment of NIAS</li> </ul>				<p>M39</p> <p>M42</p> <p>M42</p>

=> See slide on expected impacts

## Task 4.1: Dissemination of the scientific results

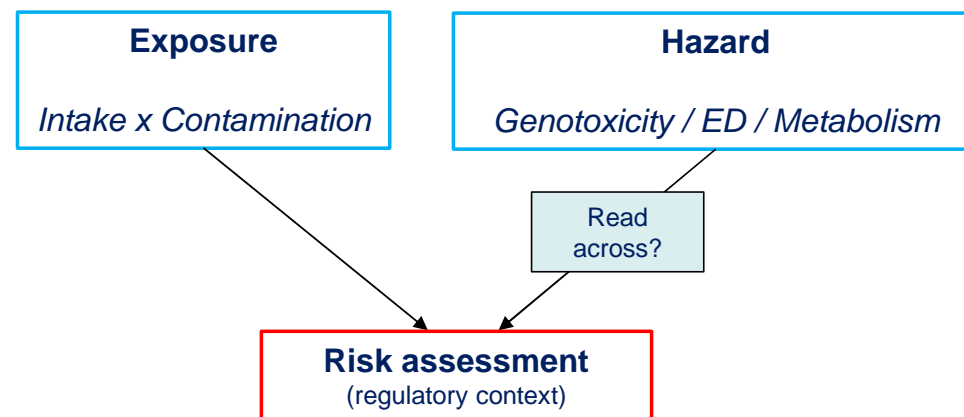
### Articles

- Genotoxicity/ED potency
- Human liver metabolism
- Prevalence of oligoesters in foodstuffs

### Symposiums

- 8 travels, ≥6 events

## Task 4.2: Provisional risk assessment



## Task 4.3: Guideline on NIAS in coatings

