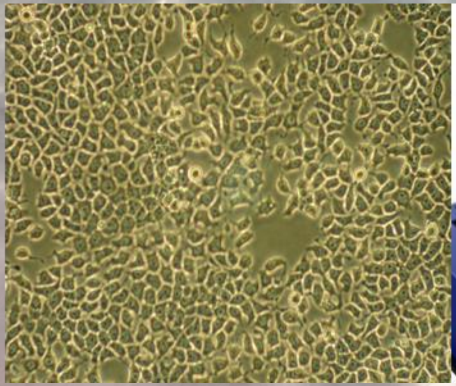
Sustainable & Safer Materials

Non-animal methods (NAMs) for toxicity testing

Dr. Peter A. Behnisch

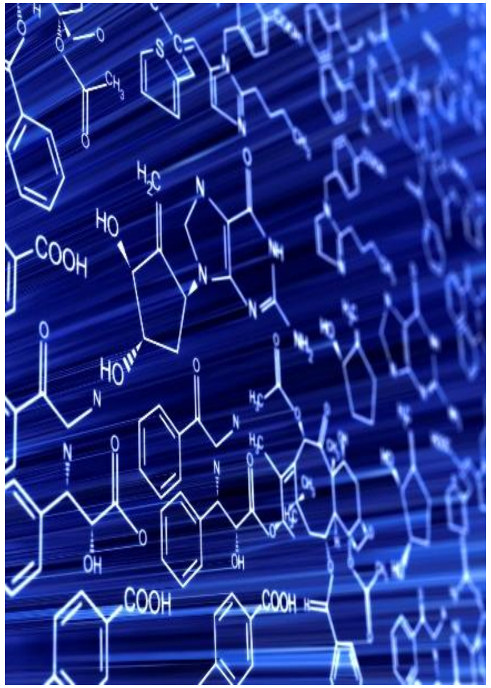
BioDetection Systems, Amsterdam



CONTENTS

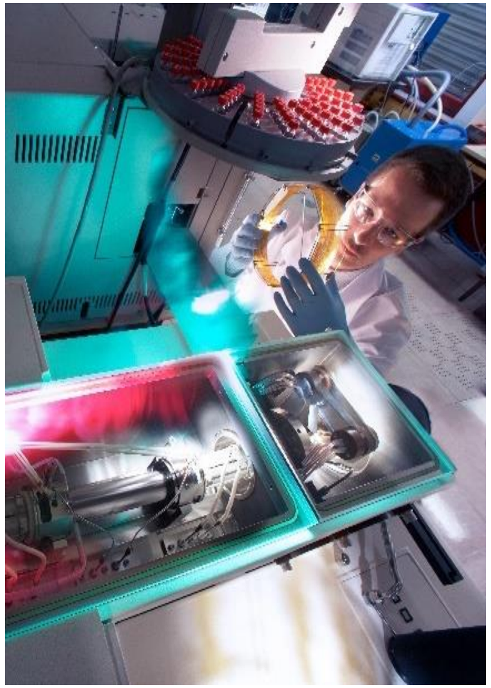
1 - The Challenges in Materials Safety

Many chemicals
(>10’000)
used in the Plastic Device



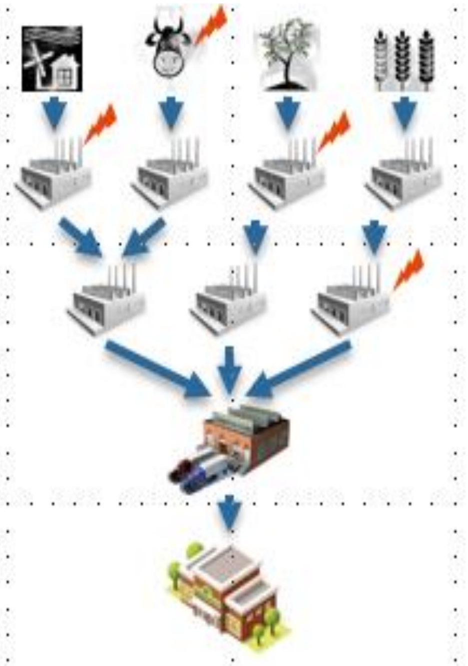
Plastic Device materials are diverse and often complex

Testing of Plastic Device are costly challenging



Lack of supra-national and harmonized regulation

Complex,
often fragile supply chains



2 - Identifying and assessing chemicals of concern

Phthalates, mineral oils, PFAS, EDCs, PAAs, PFCs, photoinitiators, BPA…

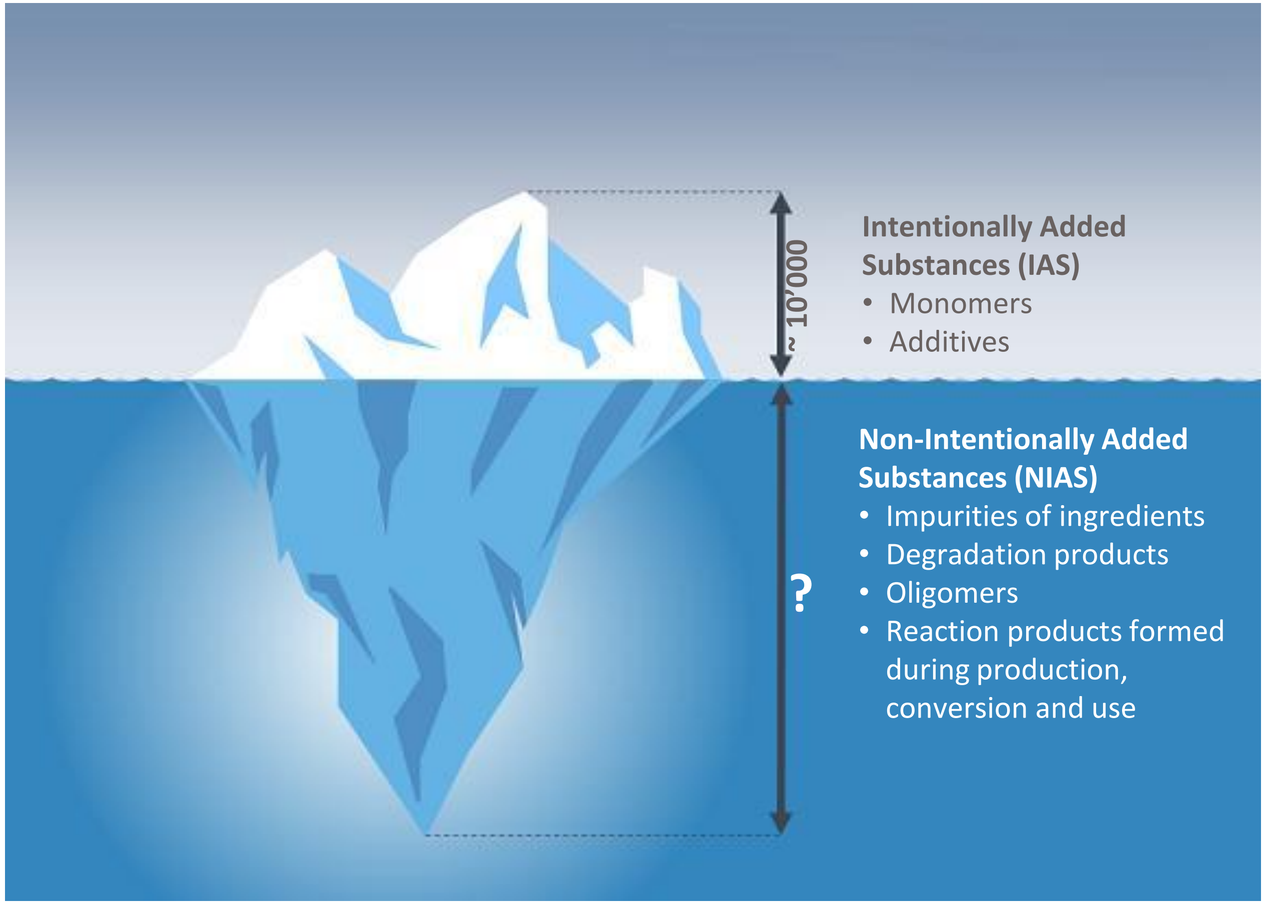
Plastics

Paper & Board

Metal coatings

Printing inks

Adhesives



3 - Non-animal methods (NAM) for toxicity testing of plastics in the circular economy

Bioactive Food Pollutants	HTPS CALUX	Pathway
C- and N-Dioxins PXDD/Fs, dl-PXBs (X= Cl, Br, F, methyl)	DR CALUX	Dioxin receptor
Carcinogenic PAHs (such as Benzo(a)pyrene)	PAH CALUX	Dioxin receptor
Estrogens, EDCs, Bisphenol A, Phthalates, Pesticides, Pharmaceuticals, cosmetics	ER CALUX	Estrogen receptor mix
Androgens, EDCs, Bisphenol A, Pesticides, Pharmaceuticals	AR CALUX	Androgen receptor
Progestins, EDCs, Anti-babypill, Pesticides, Pharmaceuticals	PR CALUX	Progesterone receptor
Glucocorticoids, EDCs, Asthma spray, Immune-suppressive agents	GR CALUX	Glucocorticoid receptor
Thyroid hormones, EDCs, Brominated flame retardants	TR CALUX	Thyroid receptor
Retinoids, Pesticides, Pharmaceuticals	RAR CALUX	Retinoic acid receptor
Obesogens, fluorinated compounds PFAAs, Anti-diabetic pharmaceuticals	PPARgamma CALUX	Peroxisome proliferatorϒ1 receptor
Obesogens, fluorinated compounds PFAAs, Anti-diabetic pharmaceuticals	PPARalpha CALUX	Peroxisome proliferatorα receptor
Pro-inflammatory cytokines	NFkappaB CALUX	NFkappaB activation
Cytotoxic/static agents, Genotoxic compounds like PAHs, Pharmaceuticals, dyes	p21 CALUX	p21 activation
Cytotoxic/static agents, Genotoxic compounds like PAHs, Pharmaceuticals, dyes	p53 CALUX	p53 transcriptional activity
Electrophiles, oxidative stress, heavy metals	Nrf2 CALUX	Nrf2 transcriptional activity
β-Catenin/ involved in development and carcinogenesis	TCF	TCF transcriptional activity
Carcinogens, UV	AP1 CALUX	AP1 transcriptional activity
Hypoxia-mediated angiogenesis	HIF1alpha CALUX	HIF1α transcriptional activity
Endoplasmatic reticulum stressors	ER stress CALUX	XBP1 transcriptional activity
Cytotoxic agents, Non-specific luciferase modulators	Cytox CALUX	Constitutive transcriptional activity

Table 1: Currently available HTPS CALUX assays

Behnisch 2012 – Food Lab International

High-through-put screening (HTPS) - human cell-based TOX

BDS laboratory (Amsterdam)

HTPS cell handling equipment

Microlab (Hamilton)

Luminometer

4 - Toxicological profiling of materials additives (such as BPA & phthalates)

Public concern about safety of consumer products

Scientists Fear Chemical in Plastic Could Be Harmful

abc NEWS

By JAIME J. HENNESSEY
July 6, 2006

From food-storage containers to disposable silverware, plasti…
are such a part of our lives that it's …
that could harm u…

Plastic chemicals 'feminise boys'

Chemicals in plastics alter the brains of baby boys, making them "more feminine", say US researchers.

Males exposed to high doses in the womb went on to be less likely to play with boys' toys like cars or to join in rough and tumble games, they found.

SPL

Male hormones drive boyish play

The University of Rochester team's latest work adds to concerns about the safety of phthalates, found in vinyl flooring and PVC sho…

The findings are reported in the Int…

…derStandard.at › Gesundheit › Leben › Umweltmedizin

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Vorsorge | Krankheit | Therapie | Leben | Se…

Weichmacher stören Hormon-Balance

Österreich verhält sich nicht EU-konform und verbietet Bisphenol A in Babyprodukten - In Kinderzimmern stecken aber noch andere gefährliche Chemikalien

Österreichische Kinder sollen künftig keine hormonaktiven Substanzen mehr aus ihren Schnullern nuckeln. Mit 1. Jänner 2011 will Gesundheitsminister Alois Stöger Bisphenol A, eine Massenchemikalie, die wie ein körpereigenes Hormon wirkt, in "kindernahen" Produkten wie Schnullern und Fläschchen verbieten. Zu finden ist BPA außer in Babyartikeln auch in den Beschichtungen von Getränke- und Konservendosen, in Trinkflaschen für Sportler, in CDs und DVDs.

BPA ist Ausgangsstoff für den transparenten und harten …

foto: ernst rose/pixelio.de

The INDEPENDENT

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News > Science

Study shows dangers of BPA chemical used in plastic packaging

Bisphenol A is used to line drinks cans and in tests affected the way genes work in the brains of laboratory rats

Are Plastic Baby Bottles Harmful?

By Laura Blue | Friday, Feb. 08, 2008

If a new report is to be believed, an entire generation of children has grown up drinking a toxic chemical from their earliest months: bisphenol A. A consortium of North American environmental and health groups released a paper Thursday showing that many major-brand baby bottles leach bisphenol A, and is now calling for a moratorium on the use of the compound — used to make polycarbonate plastic — in food and beverage containers.

Researchers tested 19 baby bottles purchased in nine …

ISO 19040-3: Hormone-like activities of plastic additives by ER CALUX

Water quality — Determination of the estrogenic potential of water and waste water — Part 3: In vitro human cell-based reporter gene assay (ISO 19040-3:2018)

Tabelle D.1 — Zusammenfassung relativer Potenzen (P_r) im Vergleich zu 17β-Estradiol für ausgewählte Verbindungen

Verbindung	U2OS-ERα	Literatur	T47Dαβ	Literatur
17β-Estradiol	1		1	
17α-Ethinylestradiol	1,3 bis 1,5	[10] [11] [25]	1,2	[12] [14] [15]
17α-Estradiol	0,1	[10] [11] [16]	0,016	[12] [14] [15]
Estron	0,02	[10] [11] [16]	0,056	[12] [14] [15]
4-Nonylphenol	5,9E-04	[10] [11]	2,3E-05	[12] [14] [15]
Dimethylphtalat			1,1E-05	[12] [14] [15]
Genistein	1,1E-04	[11]	6,0E-05	[12] [14]
o,p-DDT	1,9E-05	[11]	9,1E-06	[12] [14]
Methoxychlor	1,8E-06	[11]	1,0E-06	[12] [14]
Bisphenol A			7,8E-06	[12] [14] [15]
Nonylphenolethoxylat			3,8E-06	[12] [14] [15]
4-Octylphenol			1,4E-06	[12] [14] [15]
Diethylphtalat			3,2E-08	[12] [14] [15]
Di-*n*-butylphthalat			1,8E-08	[12] [14] [15]
Equol	7,6E-04	[10] [11]		
Norethynodrel	0,015	[10]		
Di(2-ethylhexyl)phthalat			> 6,0E-07	[12] [14] [15]
Estriol	0,017	[11]		

5 - Building and implementing strategies with the industry: e.g. AkzoNobel

ACS Sustainable Chemistry & Engineering

pubs.acs.org/journal/ascecg

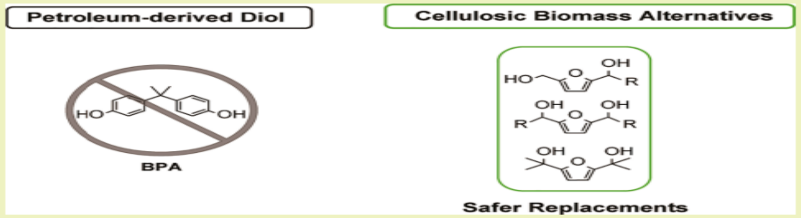
Letter

Novel Biobased Furanic Diols as Potential Alternatives to BPA: Synthesis and Endocrine Activity Screening

Catherine A. Sutton, Alexander Polykarpov, Keimpe Jan van den Berg, Alexander Yahkind, Linda J. Lea, Dean C. Webster,* and Mukund P. Sibi*

Cite This: https://dx.doi.org/10.1021/acssuschemeng.0c08207 Read Online

ABSTRACT: A series of asymmetric and symmetric diols were prepared in high yields from biomass-derived feedstocks 5-hydroxymethyl furfural (HMF) and 2,5-diformyl furan (DFF) as potential replacements for bisphenol A (BPA). The diols were screened for estrogenic, androgenic, antiandrogenic, and antithyroid activities in reporter gene assays. Several of the low molecular weight asymmetric diols did not exhibit activity in any of the assays and thus have promise as potentially more sustainable alternatives to BPA.



KEYWORDS: *Furanic diols, Bisphenol A replacement, Biomass, 5-Hydroxymethyl furfural, Endocrine activity*

Table 1. Endocrine Activity of Asymmetric Furanic Diols vs BPA[a]

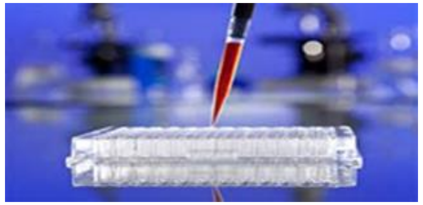
Entry	ER-α-CALUX[b]	AR CALUX[b]	Anti-AR CALUX[c]	Anti-TRβ-CALUX[c]
BPA	0.075	ND	0.013	27
3	NA	NA	NA	NA
5a	NA	NA	NA	NA
5b	NA	NA	NA	NA
5c	410	NA	78	300
5d	710	NA	120	360
5e	NA	NA	49	NA
5f	62	NA	40	110
5g	9.65	NA	NA	NA
5h	53	NA	50	71

5 - Building and implementing strategies with the industry: e.g. Nestle & PPG

APPROACH OF NESTLÉ RESEARCH CENTER
FOR METAL PACKAGING COATINGS
(VERSION 1.0 NOV_2017)

Migration or full material testing

Bioassays battery



BDS

Safety by design plastic testing

Safety by design: bioassay data on R&D food contact materials (FCM)

Biological activity	FCM 1	FCM 2	FCM 3	FCM 4	FCM 5
Hormone receptors	active		active		
	active	active			
	active	active	active		
AhR	active	active	active	active	active
Genotoxicity	active	active			
Cytotoxicity	active	active	active	active	
AMES	*	*	-	-	-

Biological activity + → -

PRIORITIZATION

Hormone Activity Results by a panel of CALUX bioassays
(Marin-Kuan, Behnisch, Szabo et al. 2023)

Erα CALUX						
Migration Sample	LAB A		LAB B		LAB C	
CALUX	LAB D	LAB C	LAB D	LAB C	LAB D	LAB C
Positive Control						
Test Coating						
Uncoated Control						
Blank						

ERα 100%

Anti-Erα CALUX						
Migration Sample	LAB A		LAB B		LAB C	
CALUX	LAB D	LAB C	LAB D	LAB C	LAB D	LAB C
Positive Control						
Test Coating						
Uncoated Control						
Blank						

Anti-ERα 92%

AR CALUX						
Migration Sample	LAB A		LAB B		LAB C	
CALUX	LAB D	LAB C	LAB D	LAB C	LAB D	LAB C
Positive Control						
Test Coating						
Uncoated Control						
Blank						

AR 100%

Anti-AR CALUX						
Migration Sample	LAB A		LAB B		LAB C	
CALUX	LAB D	LAB C	LAB D	LAB C	LAB D	LAB C
Positive Control						
Test Coating						
Uncoated Control						
Blank						

Anti-AR 75%

Concordance

No Concordance

Concordance Observed for 44 out of 48 Samples

Toxicological profiling of plastic additives for safe recycling practices

compound	Cytotox10%	ERa	ERa+S9	ERa-anti	ERb	ERb-anti	AR	AR-anti	PR	PR-anti	GR	GR-anti	TRb	RAR	LXR	PXR	PPARa	PPARg	DR	PAH	Hif1a	TCF	AP1	ESRE	NFkB	Nrf2	p21	p53 GENTOX	p53 S9 GENTOX
Di(2-ethylhexyl)phthalate		-4.0								-5.2						-6.4													
Di-n-octyl phthalate																													
monoethylhexyl phtalate	-3.5							-4.5									-5.5	-4.7											
diisodecylphthalate		-4.4			-4.2																								
diisononylphthalate																												-3.0	
Dicyclohexylphthalate	-4.5	-5.3								-5.4		-5.1				-6.7													
Diethylphthalate	-3.5	-4.3						-5.0		-4.3																			
Diisobutyl phthalate	-4.0	-5.7						-5.3		-5.5																			
Dibutylphthalate	-4.5	-5.2						-5.5		-5.5																			
Di(n-hexyl)phthalate	-3.5	-5.0						-5.0		-5.5		-4.5							-4.0				-4.2						
Butyl benzyl phthalate	-3.9	-6.4			-4.4			-5.6		-5.5									-3.7										
di(2-ethylhexyl)adipate																													
Benzophenone	-3.5	-5.2						-6.0		-4.8																			
Etyl paraben	-3.0	-5.2			-5.2			-5.0		-4.0																		-3.5	
4-tert-octylphenol	-5.5	-7.2			-8.5			-6.4		-6.1						-6.0													
4-n-octylphenol	-4.7	-6.2						-5.6		-5.3																			
Nonylphenol	-4.9	-5.1			-5.6			-6.5		-5.5														-4.6					
4-Cumylphenol	-4.2	-7.0	-6.4		-7.0			-6.7		-6.1		-4.5																	
p-(tert-pentyl)phenol	-4.0	-7.7						-6.3		-5.9																			
Diphenyl-p-phenylenediamine	-4.0	-5.5						-5.2		-5.4																			
Bisphenol A	-4.0	-7.3			-6.8			-6.8		-5.5		-4.5												-4.3					
Bisphenol A-dimethacrylate		-6.6			-6.5			-6.0		-5.5						-5.3												-4.7	
Bisphenol F		-6.6			-6.7			-5.4		-4.8						-4.3								-4.7				-4.5	-3.3

FDCA - little or no endocrine effects

- biobased building block to replace terephthalate in PET

activity - +

Green chemistry approach to select phthalate alternatives

Compound	Cytotox	ERa	AR-anti	PR-anti	GR-anti	TRb-anti	PPARa	PPARg	AhR	AP1	ESRE	Nrf2	p53 GENTOX
Dimethyl phthtalate	-	-	-4.7	-3.6	-	-	-	-	-	-	-	-	-
Dimethyl-2,5-furandicarboxylate	-	-	-	-	-	-	-	-	-	-	-	-	-
Diethyl phthalate	-3.5	-4.0	-5.0	-4.3	-	-	-	-	-	-	-	-	-
Diethyl-2,5-furandicarboxylate	-	-	-	-	-	-	-	-	-	-	-	-	-
Diisobutyl phthalate	-4.5	-5.3	-5.0	-5.0	-	-	-	-	-	-	-	-	-
Diisobutyl-2,5-furandicarboxylate	-	-4.3	-	-	-	-	-	-	-	-	-	-	-
Di(2-ethylhexyl) phthalate	-	-3.9	-	-	-	-	-	-	-	-	-	-	-
Di(2-ethylhexyl)-2,5-furandicarboxylate	-	-	-	-	-	-	-	-	-	-	-	-	-
Diisodecyl phthalate	-	-	-	-	-	-	-	-	-	-	-	-	-
Diisodecylfuran-2,5-dicarboxylate	-	-	-	-	-	-	-	-	-	-	-	-	-

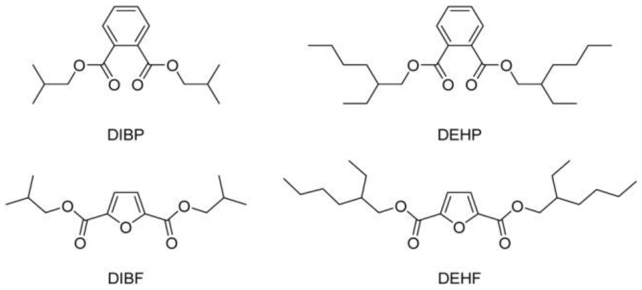
activity

Can we select promising bio-based alternatives for phthalates, with reduced endocrine activity?

- ***Furan-based counterparts largely lack endocrine activity***

Phthalate dialkyl ester

Furan-based counterpart



Van Vugt-Lussenburg et al (2020) Green Chemistry 22, 1873-1883

ROYAL SOCIETY OF CHEMISTRY

Unknown chemicals “features” in non-target analysis – how to tackle them?

Combination of chemical compound with effect-based analysis!

Testing of plastic from packaging, toys and medical devices (Kirchnawy et al 2019)

More than 500 samples of different articles

Food packaging (bottles, cups, microwave packaging, caps), granulates, films,…), Toys (plastic, textile, wood), Medical Devices (implants, infusion bags,…)

Samples included various materials:

- Composite films, food cartons
- Polystyrene, Polyolefins, PET
- Paper board
- Metal cans
- Textile and wood toys
- PVC-toys

Testing of plastic from packaging, toys and medical devices (Kirchnawy et al 2019)

- >70% of all tested samples: no endocrine activity
- < 25% of all tested sample: estrogen activity
  - Detected activity is mostly much lower than the activities previously found in mineral water (<7 ng/L EEQ)
  - < 2%: activities between 50 – 100 ng/L EEQ
- Plastic Food Packaging: lower estrogen activity than would be expected based on previous studies
- Toys, Medical Device: more endocrine activity than in food packaging

Wake-up call by Martin Wagners team:
In vitro toxicity of Bioplastics and Plant-based materials
(Zimmermann et al 2020)

Environment International 145 (2020) 106066

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

ELSEVIER

Are bioplastics and plant-based materials safer than conventional plastics? *In vitro* toxicity and chemical composition

Lisa Zimmermann[a,*], Andrea Dombrowski[a], Carolin Völker[b], Martin Wagner[c]

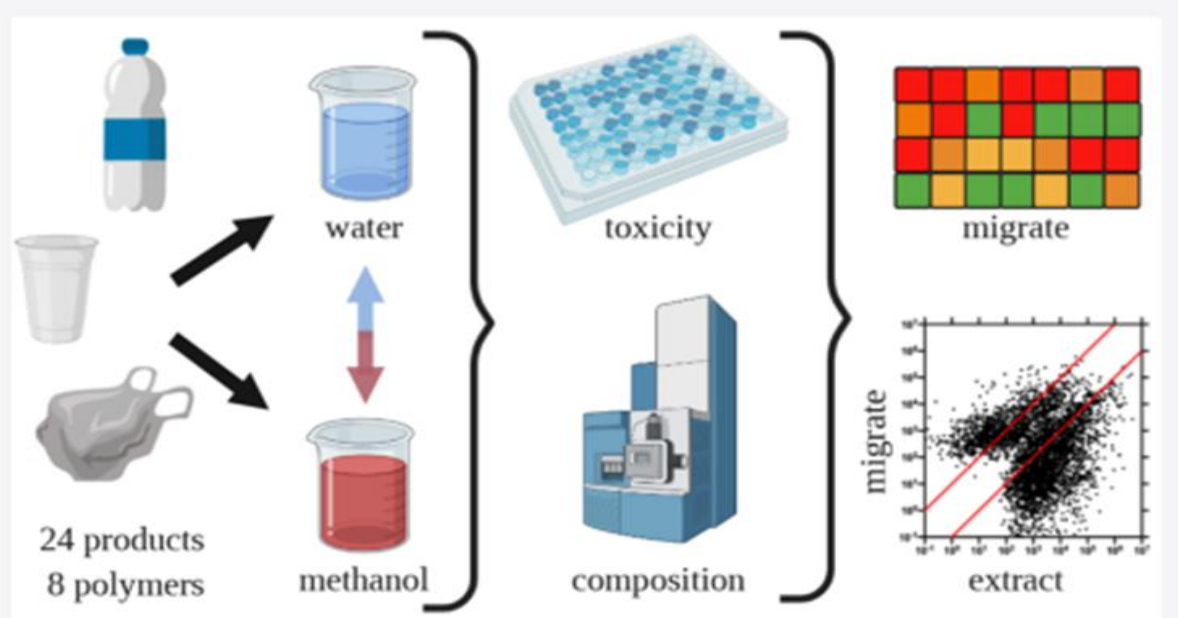


Table 1
Bioplastics and plant-based materials analyzed in this study and total number of chemicals features detected by UPLC-QTOF-MS/MS. FCM: Indication that material is suitable for food contact, Type: Raw material (RM), final product (P).

Plastic category	Sample and plastic type	Plastic product	FCM	Type	Number of detected features
Bio-based, biodegradable	PLA 1	Single-use drinking cup	+	P	3755
	PLA 2	Disposable cutlery	+	P	3479
	PLA 3	Film	+	P	8648
	PLA 4	Food tray	+	P	6465
	PLA 5	Coffee capsule	+	P	6121
	PLA 6	Bag for foodstuff	+	P	17,224
	PLA 7	Single-use bottle	+	P	3002
	PLA 8	Film		P	10,958
	PLA 9	Pellet	+	RM	3667
	PLA 10	Pellet		RM	880
	PHA 1	Pellet		RM	614
Petroleum based, biodegradable	PBS 1	Plastic bar		RM	3864
	PBS 2	Food tray	+	P	10,959
	PBAT 1	Waste bag	+	P	15,843
	PBAT 2	Pellet	+	RM	9161
Plant-based	Starch 1	Disposable cutlery	+	P	1065
	Starch 2	Bag for foodstuff	+	P	18,198
	Starch 3	Film		P	15,770
	Starch 4	Film	+	P	16,857
	Starch 5	Pellet	+	RM	9118
	Starch 6	Pellet	+	RM	8325
	Starch 7	Waste bag	—	P	20,965
	Starch 8	Film		P	11,901
	Cellulose 1	Tea bag wrapping	+	P	14,456
	Cellulose 2	Chocolate wrapping	+	P	3378
	Cellulose 3	Cigarette filter	—	P	15,719
	Cellulose 4	Pellet	+	RM	2953
	Cellulose 5	Bag for foodstuff	+	P	20,416
	Cellulose 6	Bag for foodstuff	+	P	14,031
	Cellulose 7	Bag for foodstuff	+	P	17,495
	Bamboo 1	Reusable coffee cup	+	P	5426
Bio-based, non-biodegradable	Bio-PE 1	Bag for foodstuff	+	P	5272
	Bio-PE 2	Wine closure	+	P	1629
	Bio-PE 3	Bag for foodstuff	+	P	n.a.[a]
	Bio-PE 4	Pellet		RM	819
	Bio-PE 5	Food tray	+	P	290
	Bio-PE 6	Film		P	928
	Bio-PE 7	Wine closure	+	P	947
	Bio-PE 8	Pellet		RM	186
	Bio-PE 9	Bag for foodstuff	+	P	19,028
	Bio-PE 10	Film	+	P	13,381
	Bio-PET 1	Reusable bottle	+	P	390
	Bio-PET 2	Box		P	5625

Combined chemical & effect-based NAMs

- In total, ca **41,000 chemical features** with 186–20,965 features were present in the individual samples.
- **80% of the extracts contained > 1000 features**, most of them unique to one sample.
- **343 priority compounds** including monomers, oligomers, plastic additives, lubricants and non-intentionally added substances were identified
- Extracts from cellulose- and starch-based materials generally triggered a **strong in vitro toxicity and contained most chemical features.**
- The **toxicological and chemical signatures** of polyethylene (Bio-PE), polyethylene terephthalate (Bio-PET), polybutylene adipate terephthalate (PBAT), polybutylene succinate (PBS), polylactic acid (PLA), polyhydroxyalkanoates (PHA) and bamboo-based materials **varied with the respective product rather than the material.**
- **Toxicity** was less prevalent and potent in raw materials than **in final products**. A comparison with **conventional plastics indicates that bioplastics and plant-based materials are similarly toxic.**

Zimmermann et al (2020). Env Intern 145, 106066

In vitro toxicity profiling of Plastic Consumer Products (Zimmermann et al 2021)

Environmental Science & Technology

pubs.acs.org/est Article

Plastic Products Leach Chemicals That Induce *In Vitro* Toxicity under Realistic Use Conditions

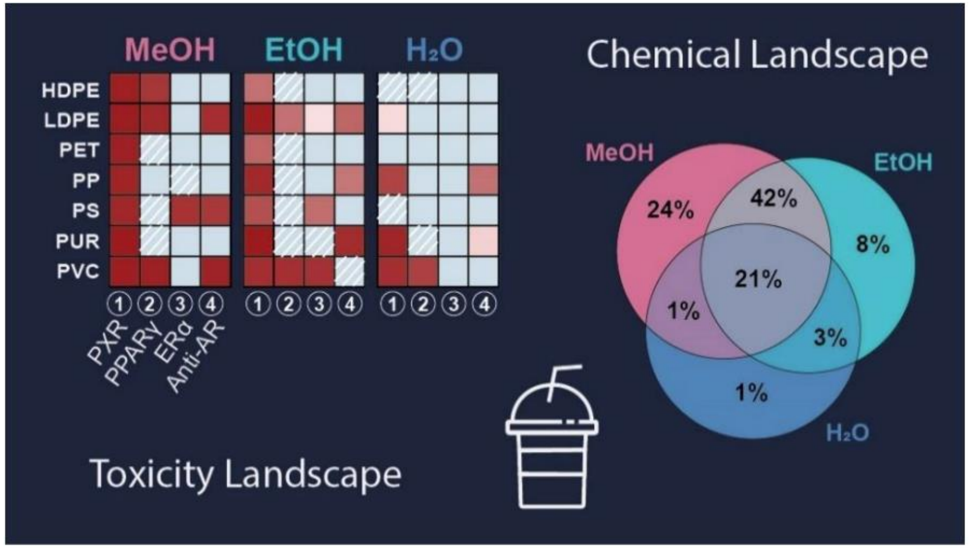
Lisa Zimmermann, Zdenka Bartosova, Katharina Braun, Jörg Oehlmann, Carolin Völker,[||] and Martin Wagner[*,||]

Cite This: *Environ. Sci. Technol.* 2021, 55, 11814–11823 Read Online

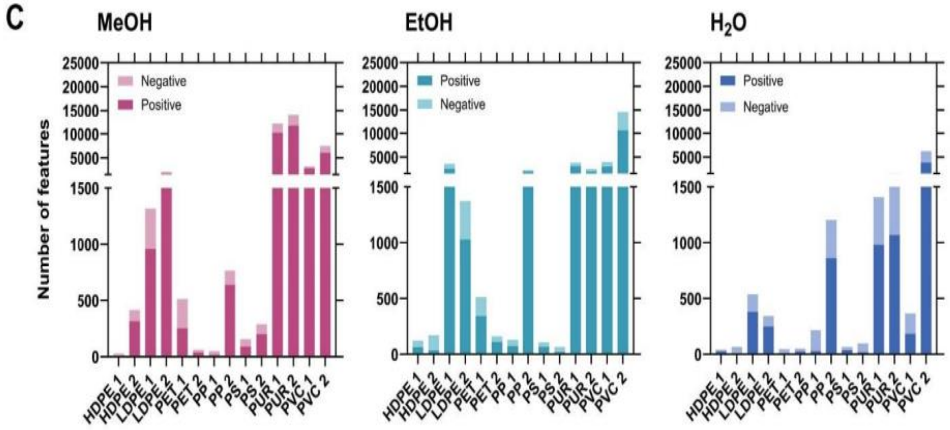
Table 1. Plastic Products Analyzed in this Study

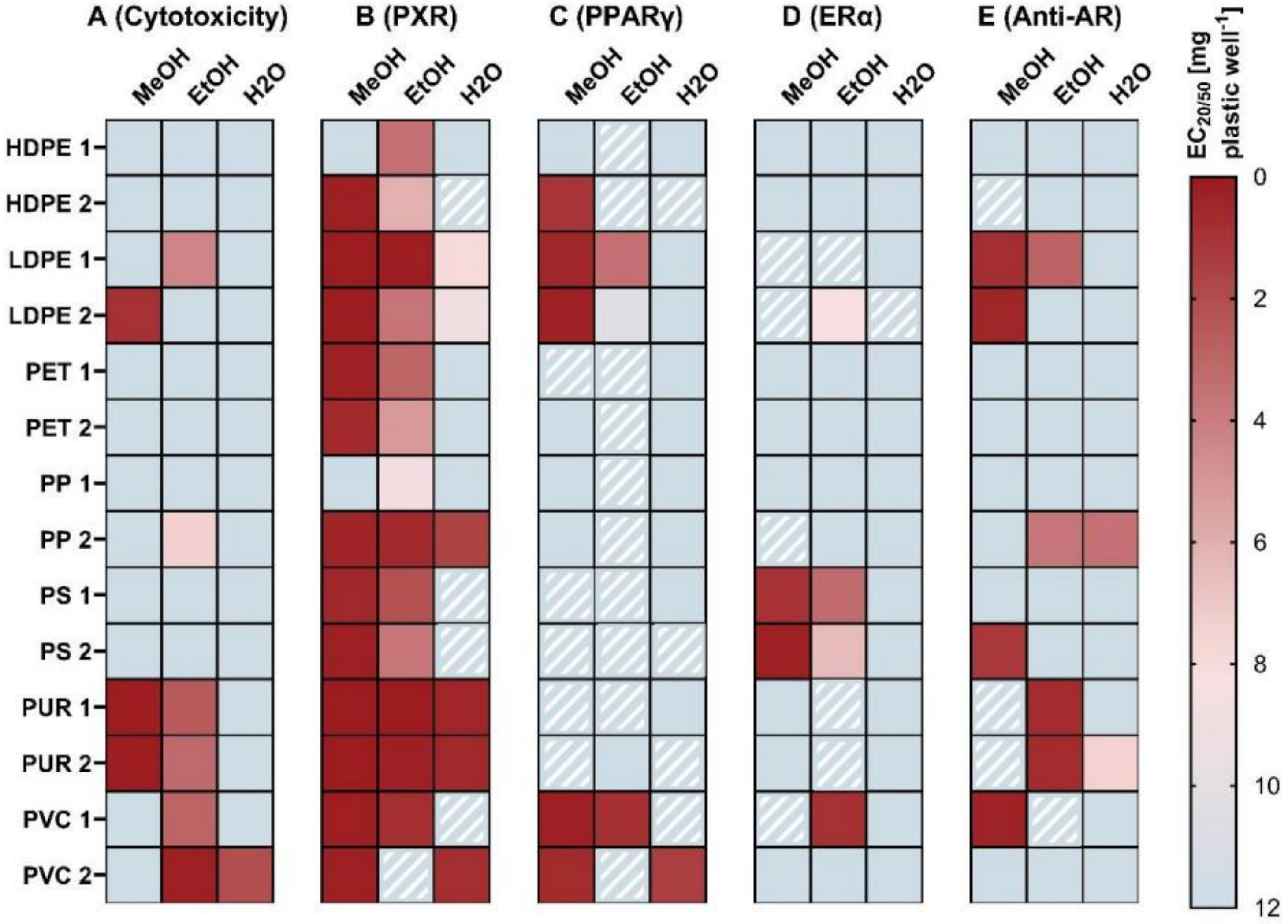
sample	plastic product	FCM[a]
HDPE 1	bin liners	
LDPE 1	lemon juice bottle	+
LDPE 2	plastic wrap	+
LDPE 3	freezer bag	+
LDPE 4	hair conditioner bottle	
PS 1	yogurt cup	+
PS 2	fruit tray	+
PS 3	vegetable tray	+
PS 4	plastic cup	+
PP 1	yogurt cup	+
PP 2	gummi candy packaging	+
PET 1	oven bag	+
PVC 1	plastic wrap	+
PVC 2	placemat	
PVC 3	pond liner	
PVC 4	floor covering	
PUR 1	scouring pad	
PUR 2	kids bath sponge	
PUR 3	acoustic foam	
PUR 4	shower slippers	
PLA 1	yogurt cup	+
PLA 2	vegetable tray	+
PLA 3	shampoo bottle	
PLA 4	coffee cup lid	+

Migration of endocrine and metabolism disrupting chemicals from plastic food packaging (Stevens et al 2024)



Keywords: endocrine disruptor, food contact material, mixture toxicity, leachate toxicity, non-target chemical analysis, nuclear receptor





PFAS in disposable food packaging and tableware: Effect-based testing by BDS

OIL-BEADING TAKEAWAY PAPER

SAMPLE ID	TOF (mg/kg dw)	TOF (µg/ dm2 dw)	6:2 FTOH (ng/g)	6:2 FTS (ng/g)	10:2 FTS (ng/g)	% identified fluorine	TTR - FITC-T4 activity (µg PFOA/g)	TTR - FITC-T4 LOQ (µg PFOA/g)
DE-PAP-KFC-17a	**770**	247	**528**	<LOQ	<LOQ	0.047	**341**	26
FastF-FR-5	**700**	215	**706**	<LOQ	<LOQ	0.068	**220**	29
FastF-FR-3	**670**	224	**192**	**39.5**	**104**	0.033	NA	-
DE-PAP-NRDS-19a	**640**	291	**234**	<LOQ	<LOQ	0.025	NA	-
FastF-FR-2	**530**	351	**219**	<LOQ	<LOQ	0.028	NA	-
DE-PAP-DDNT-20a	**510**	270	**194**	<LOQ	<LOQ	0.026	NA	-
FasF-UK-5a	**480**	157	**16.9**	<LOQ	<LOQ	0.0024	39	19
CZ-FCM-KFC-06	**480**	134	**634**	<LOQ	<LOQ	0.090	69	33
CZ-FCM-MCD-01b	**470**	176	**335**	<LOQ	<LOQ	0.048	**52**	16
FastF-UK-2	**440**	177	<LOQ	<LOQ	**34.4**	0.0050	60	30
CZ-FCM-BB-01b	**400**	400	**345**	<LOQ	<LOQ	0.059	NA	-
FastF-UK-4	**390**	125	**248**	<LOQ	<LOQ	0.043	NA	-
DE-PAP-MCD-26	**370**	159	**132**	<LOQ	<LOQ	0.024	**180**	26

6 - International strategies for safer life cycle assessment LCA

(UNEP 2024)

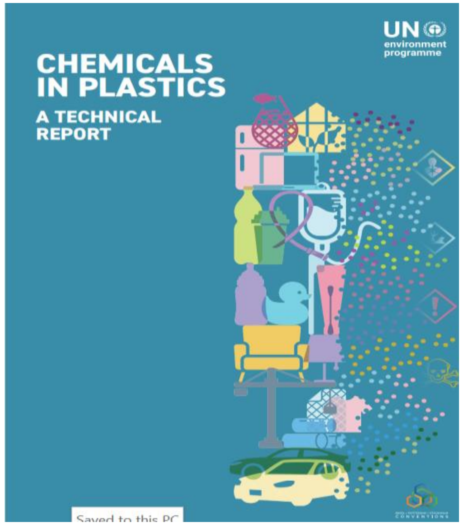
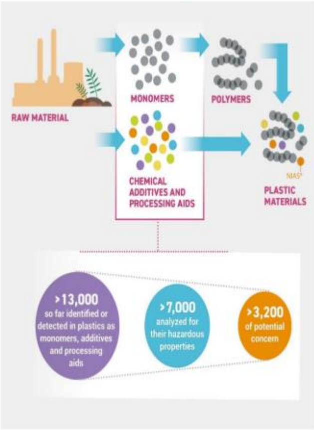


Figure 3. A simplified illustration of the production of plastics from raw materials, and an overview of chemicals that may have been used in plastic production or have been detected in plastics.



*NIAS = non-intentionally added substances, including (1) break-down products of polymers, additives and other chemicals in plastics, (2) impurities, (3) contaminants from processing such as production and recycling, and (4) reaction byproducts.

Chemicals of concern in plastics are found across various sectors and products value chains

Table 4. A selection of *in vitro* bioassays that have been used in NIAS research (Koster *et al.* 2016).

Potential endocrine activity	Cytotoxicity	Genotoxicity/potential carcinogenicity
Oestrogen receptor (ER) redistribution Androgen receptor (AR) redistribution	Cell Organelle Health (COH); end points: DNA content, cytochrome C, mitochondrial membrane potential, RNA synthesis kinetic inhibition.	Indicator assays for genotoxicity (PARP, GADD45,...), Comet-FPG assay
Transcriptional activation assay Oestrogen receptor (ER) (anti) Androgen receptor (AR) Glucocorticoid receptor (GR) Progesterone receptor (PR) Thyroid recepted (TR) Peroxisome Proliferator Activated Receptor (PPARγ)	Cell Proliferation and Cell Death (CPD); end points: apoptosis – caspase3, p53; DNA content, DNA proliferation – BrdU	Mutagenicity test (Ames test, mammalian cell gene mutation tests, micronucleus (MN) test) Potential carcinogenicity Cell Transformation Assay (detection of both geno- and non-genotoxic carcinogens)
H295R Steroidogenesis assay (changes in hormone production)		

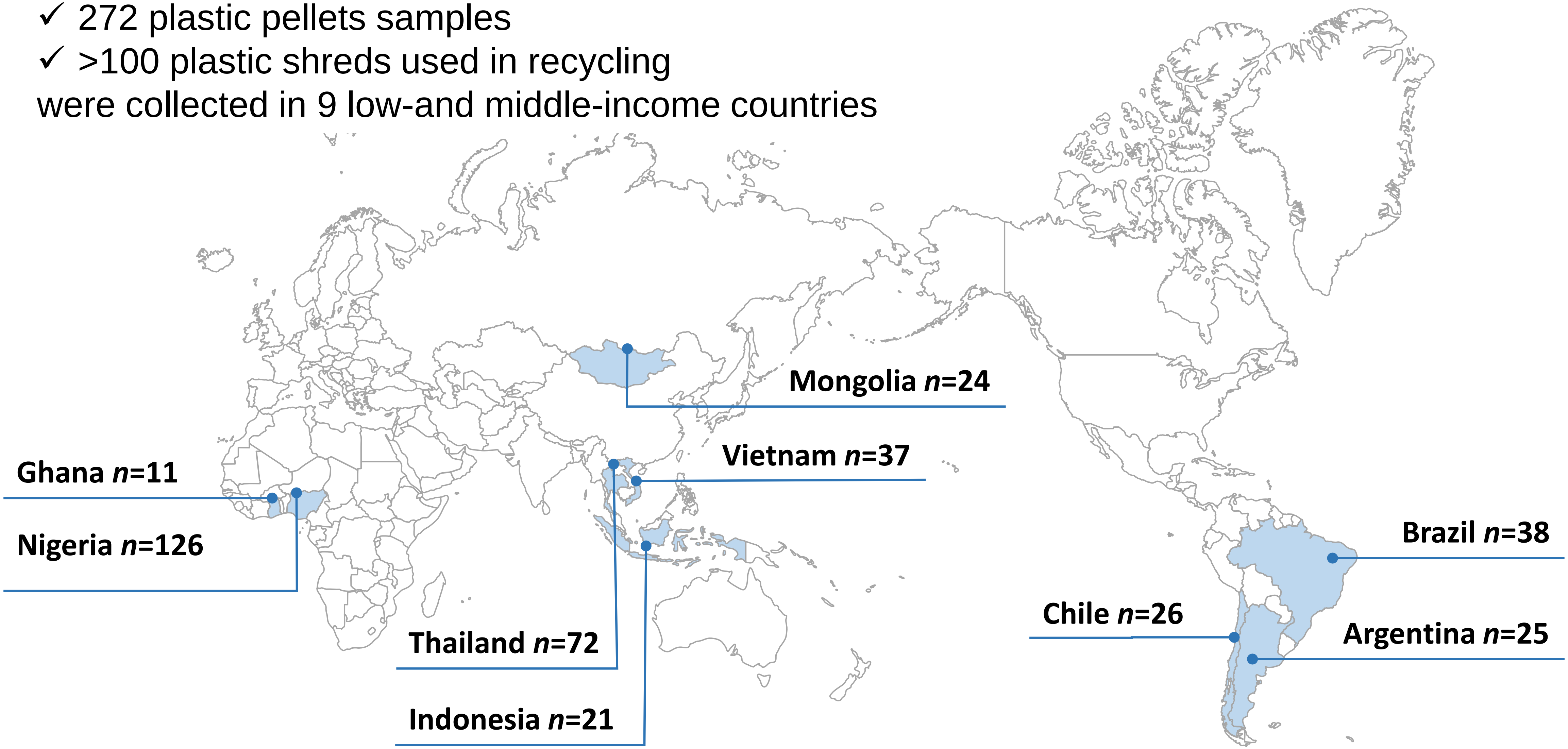
SOLUTIONS to move FORWARD

Update regulatory testing guidelines, for instance by including rapid and cost-effective approaches such as bioassays and computational tools. This serves as a prerequisite for research to fill existing knowledge gaps, e.g., regarding the mixture toxicity of leachates from plastics or the toxicity and bioaccumulation potential of hydrophobic chemicals.

One option to assess the overall toxicity of chemicals released from plastic products is to test the overall extract/leachate using *in vitro* bioassays, which are rapid and cost-effective (Groh and Muncke 2017), and use other screening technologies that measure relevant toxicological effects such as cytotoxicity, genotoxicity and endocrine effects (Table 4) (Koster *et al.* 2016). The AOP approach (see section 4.1.2) can

Global Plastic pellets monitoring (UNEP 2024)

✓ 272 plastic pellets samples
✓ >100 plastic shreds used in recycling
were collected in 9 low-and middle-income countries



High through-put extraction of plastic

A) THF/hexane:

- 0.5 gr plastic, milling,
- add 10 ml THF, shake 20 min,
- add dropwise 2 x 10 ml hexane, combine,
- add 50 ul DMSO, evaporate to a final volume of 50 ul.

B) 50% Ethanol/Water:

- 0.5 gr plastic, milling,
- add 10 ml EtOH/Water (50%), put in oven at 60 ° C for 3 days
- add 50 ul DMSO, evaporate to a final volume of 50 ul.

C) 20% Ethanol/Water:

- 0.5 gr plastic, milling,
- add 10 ml EtOH/Water (20%), put in oven at 40 ° C for 1 day
- add 50 ul DMSO, evaporate to a final volume of 50 ul.

In vitro toxicity profiling by CALUX panel: Overview all samples

Sample code	CALUX bioassay				
code	Cytotox (µg Tributyltin acetate eq./ gram product)	P53	PAH	ERa	Anti-AR
N3 Nigeria: THF/hexane	41	LOQ (<0.28)	2000	LOQ (<0.13)	LOQ (<8.3)
N9 Nigeria: THF/hexane	32	LOQ (<0.28)	610	LOQ (<0.13)	LOQ (<8.3)
N22 Nigeria PVC: THF/hexane	1.6	LOQ (<0.11)	45000	5.7	390
N24 Nigeria PP: THF/hexane	16	LOQ (<0.084)	1500	LOQ (<0.034)	12
N24 Nigeria PP: 50% ethanol/ H_20	LOQ (<0.34)	LOQ (<0.0096)	3200	0.16	10
N22 Nigeria PVC: 20% ethanol/ H_20	1.1	LOQ (<0.096)	470	0.044	2.8
N9 Nigeria: 50% ethanol/ H_20	0.34	LOQ (<0.029)	600	0.47	14
N3 Nigeria: 50% ethanol/ H_20	0.55	LOQ (<0.027)	7000	LOQ (<0.044)	LOQ (<4.0)
UNEP046_3/Arg 1_3: 20% ethanol/ H_20	LOQ (<0.87)	LOQ (<0.026)	1.8	LOQ (<0.071)	LOQ (<9.3)
UNEP070_4_3/IND13_4: 20% ethanol/ H20	LOQ (<0.94)	LOQ (<0.026)	LOQ (<0.86)	LOQ (<0.074)	LOQ (<9.3)
UNEP065_4/Viet 2_4: 20% ethanol/ H_20	LOQ (<0.94)	LOQ (<0.026)	LOQ (<0.86)	LOQ (<0.074)	LOQ (<9.3)
UNEP064_6/Arg 1_6: 50% ethanol/ H_20	LOQ (<0.87)	LOQ (<0.026)	LOQ (<0.86)	LOQ (<0.079)	LOQ (<7.3)
UNEP070_6/IND13_6: 50% ethanol/ H20	LOQ (<0.94)	LOQ (<0.029)	14	LOQ (<0.091)	45
UNEP071_6/IND24_6: 50% ethanol/ H_20	LOQ (<0.90)	LOQ (<0.027)	LOQ (<0.61)	LOQ (<0.087)	LOQ (<8.8)
UNEP065_6/Viet 2_6: 50% ethanol/ H_20	LOQ (<0.90)	LOQ (<0.027)	2.8	LOQ (<0.079)	LOQ (<11)
UNEP066_6/Viet18_6: 50% ethanol/ H_20	LOQ (<0.90)	LOQ (<0.027)	12	LOQ (<0.078)	LOQ (<11)
UNEP067_6/Viet 22_6: 50% ethanol/ H_20	LOQ (<0.92)	LOQ (<0.028)	1.4	LOQ (<0.077)	LOQ (<9.9)
UNEP064_7/Arg 1_7: THF/hexane	LOQ (<0.85)	LOQ (<0.027)	490	0.17	LOQ (<9.2)
UNEP070_7/IND13_7: THF/hexane	LOQ (<0.87)	LOQ (<0.027)	LOQ (<0.89)	LOQ (<0.091)	18
UNEP071_7/IND24_7: THF/hexane	LOQ (<0.98)	LOQ (<0.027)	41	LOQ (<0.095)	LOQ (<11)
UNEP065_7/Viet 2_7: THF/hexane	LOQ (<0.94)	LOQ (<0.027)	1.3	LOQ (<0.095)	LOQ (<11)
UNEP066_7/Viet 18_7: THF/hexane	LOQ (<0.94)	LOQ (<0.027)	LOQ (<1.2)	0.30	LOQ (<9.1)
UNEP067_7/Viet 22_7: THF/hexane	LOQ (<0.98)	LOQ (<0.028)	130	LOQ (<0.087)	11
BW_2: 20% ethanol/ H20	LOQ (<0.92)	LOQ (<0.028)	LOQ (<0.91)	LOQ (<0.075)	LOQ (<9.9)
BW_6: 50% ethanol/ H20	LOQ (<0.92)	LOQ (<0.028)	LOQ (<0.91)	LOQ (<0.084)	LOQ (<7.7)
BW_7: THF/hexane	LOQ (<0.92)	LOQ (<0.028)	LOQ (<0.85)	LOQ (<0.077)	LOQ (<9.9)

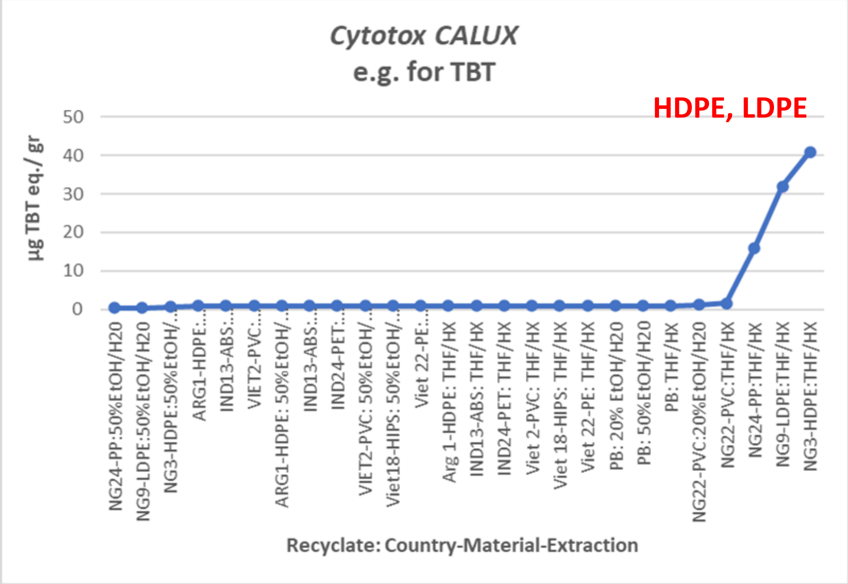
IPCP International Panel on Chemical Pollution

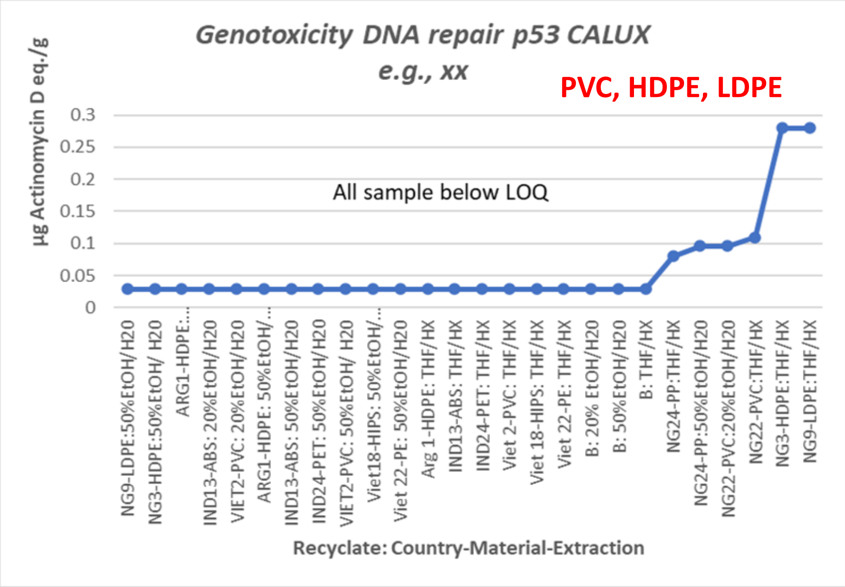
UN environment programme

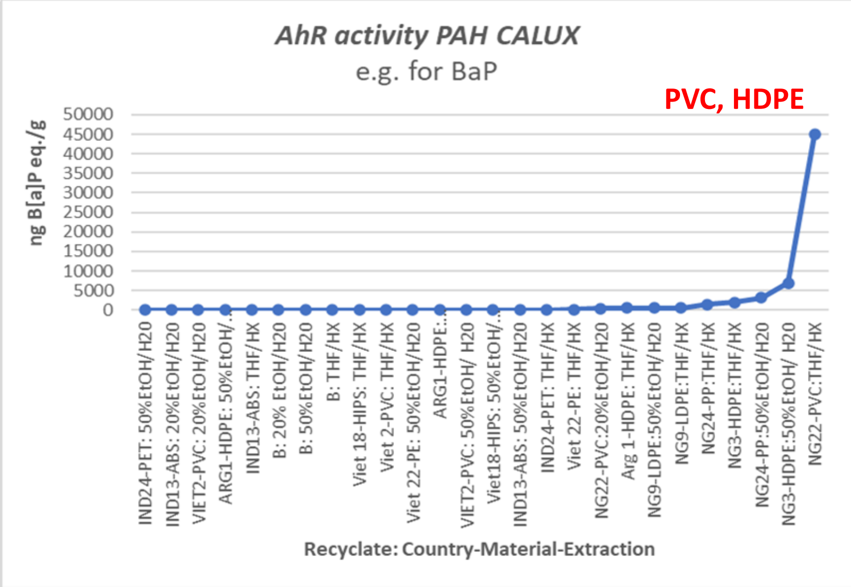
Overview in vitro toxicity profiling of plastic

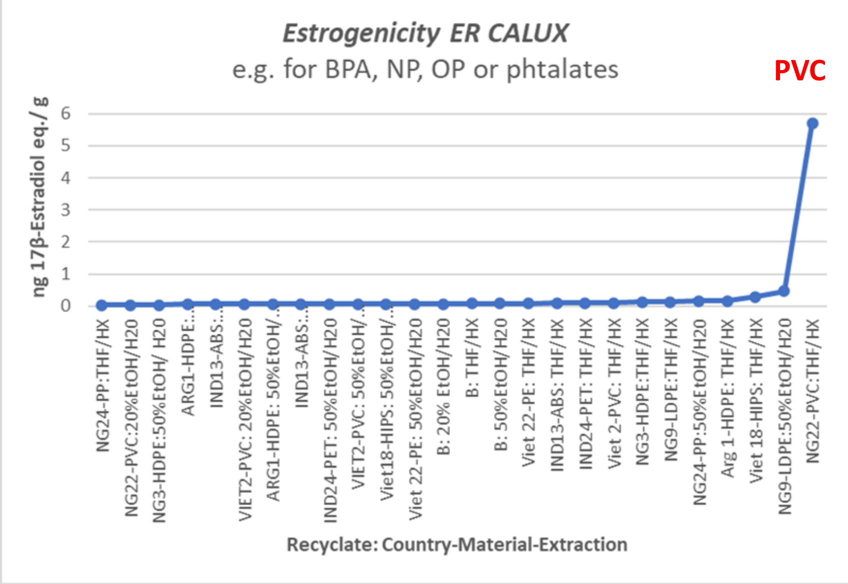
STOCKHOLM CONVENTION

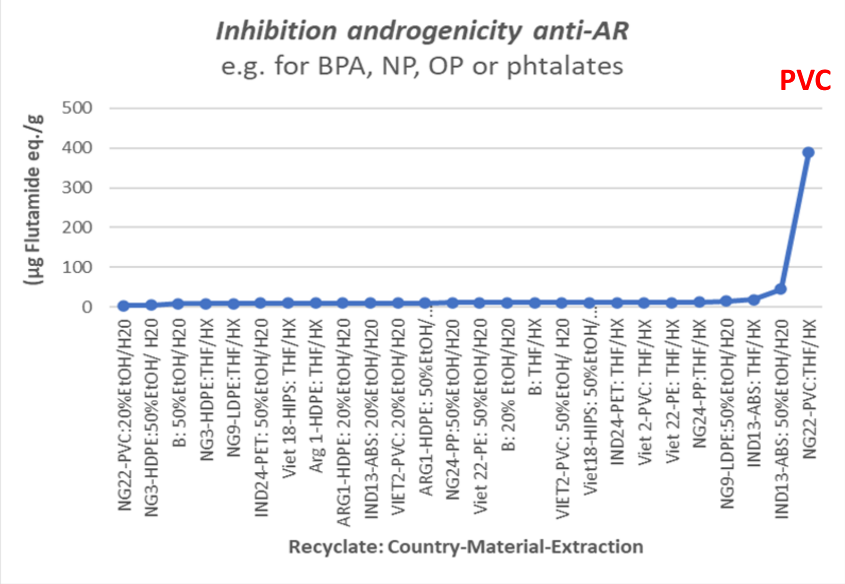
gef











- *PVC samples most toxic*

- *THF/hexane extraction most toxic*

- *PAH-like activity of highest concern*

- *More testing needed for EDC properties (ER, anti-AR)*

- *additional toxic mode of actions need to be added (stable AhR, anti-PR, anti-TR and TTR-TR)*

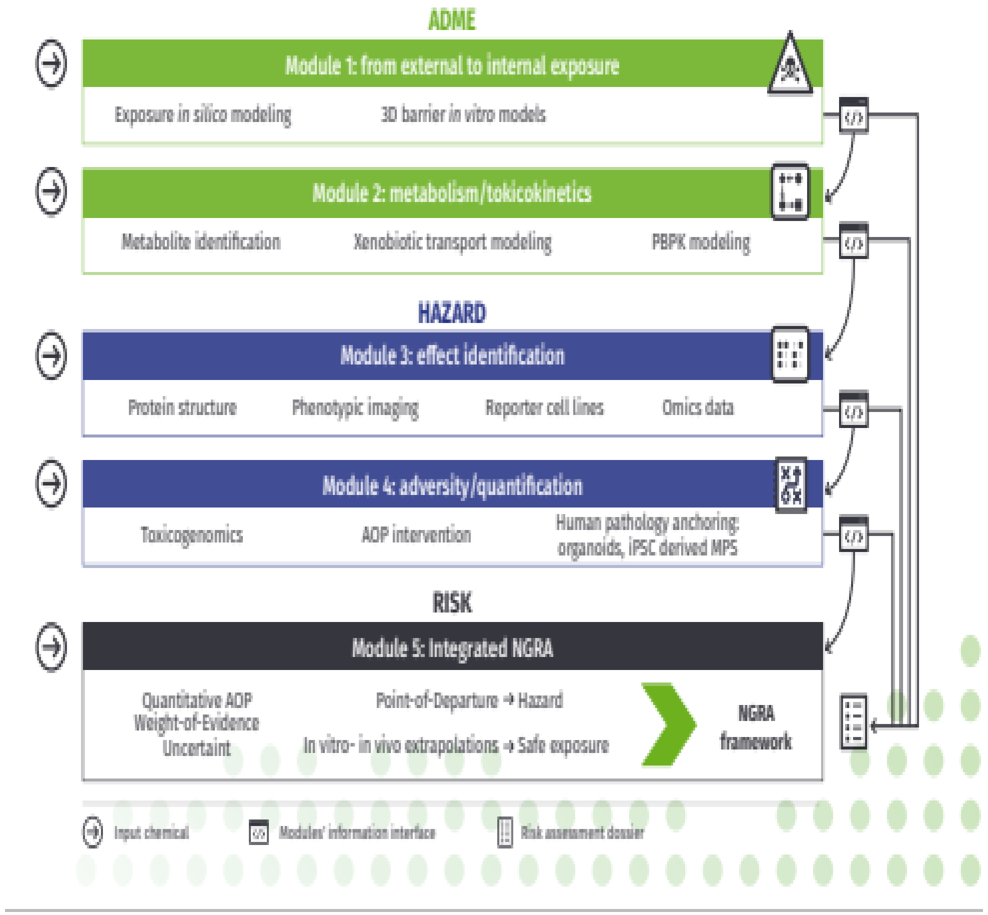
Current EU projects BDS involved: RISKHUNTER3R
Safe & Sustainable by Design

RISK HUNT3R

RISK assessment of chemicals integrating HUman centric Next generation Testing strategies promoting the 3Rs

www.risk-hunt3r.eu

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 964537.



Current EU project BDS involved CHAMPION
Test strategies for safer bio-based polymers

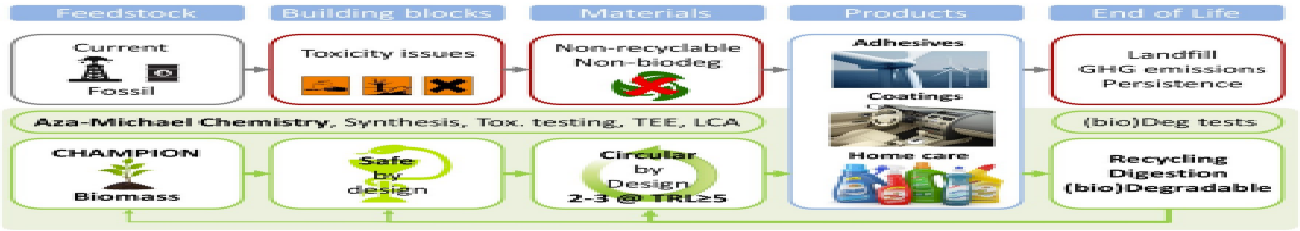
www.champion-project.eu

CHAMPION
circular bio-based polymers

Circular High-performance Aza-Michael Polymers as Innovative materials Originating from Nature

CHAMPION aims to replace conventional polymers with novel and sustainable bio-based polymers for their application in coatings, textiles, home care uses and structural adhesives.

The majority of conventional polymers are not fit for recycling and end up being incinerated or landfilled. Novel CHAMPION bio-based polymers, resulting from the aza-Michael addition reaction, are expected to be suitable replacements for polymers used in resistant kitchen counter coatings, laundry detergents and other homecare products, car interior surfaces, and structural adhesives. Recovery, chemical recycling and organic recycling are the end-of-life options planned for the design of products using CHAMPION polymers.



CHAMPION Objectives

- Produce a library of + 50 novel bio-derivable and bio-degradable materials
- Test CHAMPION polymers for home care formulation additives, structural adhesives, coatings and automotive interior surfaces
- Increase resource efficiency and reduce GHG emissions with novel polymers
- Evaluate polyester candidates in environmental, social and economic terms
- Establish an innovative testing strategy to evaluate toxicological safety

PARTNERS

University of York, SQ consult, BDS, PDC, OWS, Scott Bader, Orineo, Wageningen University & Research, VTT, Stahl, Circa, nova Institute, Unilever, AVA Biochem

Take home message for
Toxic-free Sustainable Materials in the Circular Economy

- For a **SAFER & SUSTAINABLE** approach complex mixtures of known and unknown chemicals in plastic and bioplastics need to be monitored by a **COMBINED** chemical AND effect-based biological toxicity screening tests (e.g., OECD TG455 and TG458)!

- **Safe design/Green chemistry** using in vitro toxicity is already in many R&D applications for all kinds of plastic materials used, but international regulatory framework is missing!

- High-throughput bioassays are used decades already for chemicals testing, why not using also for **safer SUSTAINABLE PLASTIC testing** following industrial and NGO global leaders…

Invitation for 14th BioDetectors Conference

Safe & sustainable materials

Happy to answer your questions

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