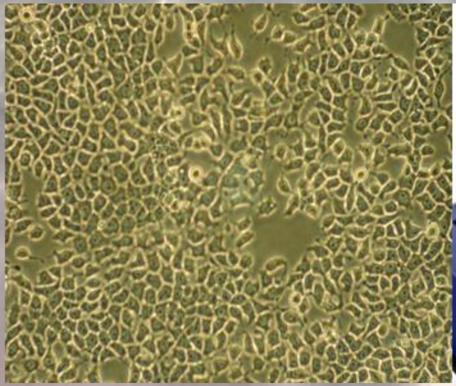
IPCP webinar, for a UNEP project, on persistent organic pollutants (POPs) and POP candidates in plastics.

Bioanalysis of POPs and EDC in plastic

RESPONSIBLE:

Dr. Peter A. Behnisch



CONTENTS

Public concern about safety of plastic

What's in YOUR blood?

First Run Features presents A DOCUMENTARY BY WERNER BOOTE

PLASTIC PLANET

Scientists Fear Chemical in Plastic Could Be Harmful

abc NEWS

By JAIME J. HENNESSEY

July 6, 2006

From food-storage containers to disposable silverware, pla...

are such a part of our lives that it's ...

that could harm u...

derStandard.at › Gesundheit › Leben › Umweltmedizin

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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

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.

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...

SPL

Male hormones drive boyish play

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

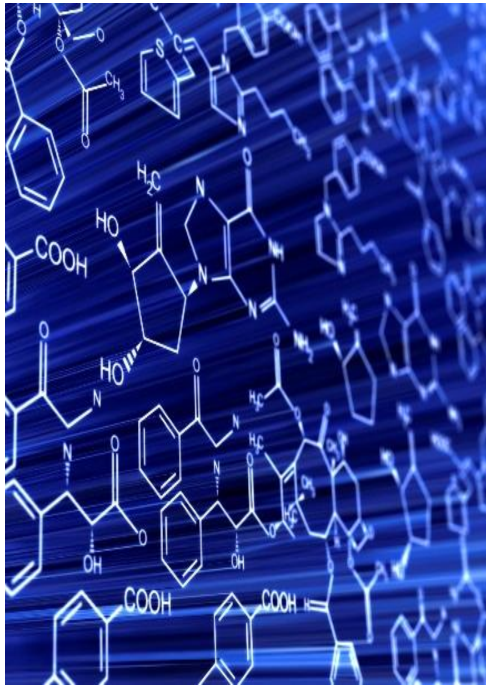
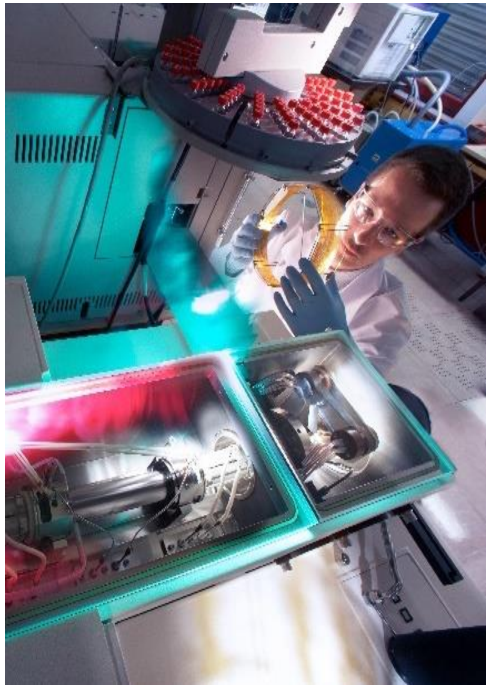
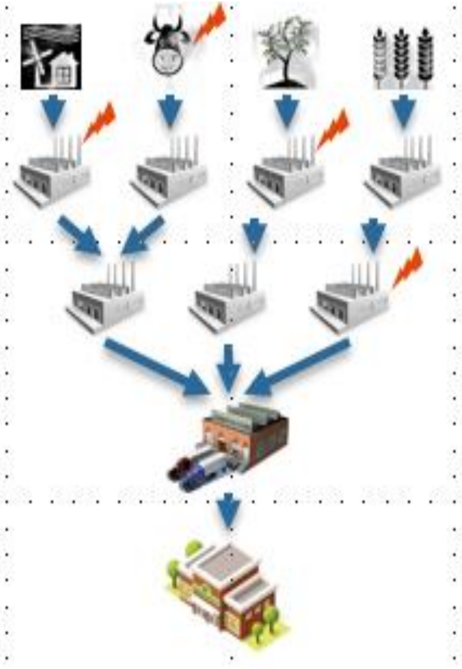
Are Plastic Baby Bottles Harmful?

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

The Challenges in Plastic 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
				

Plastics and Chemical of Concern

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

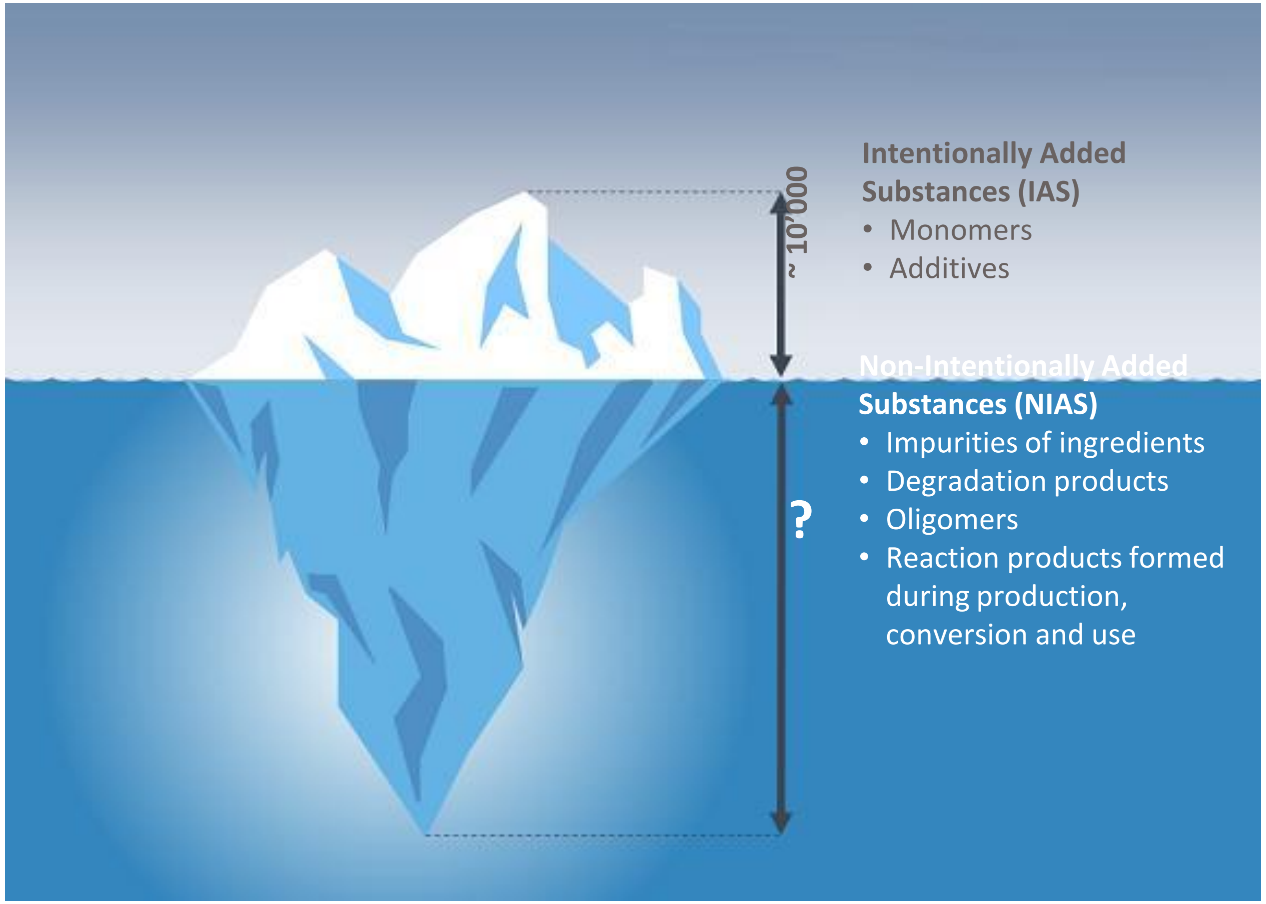
Plastics

Paper & Board

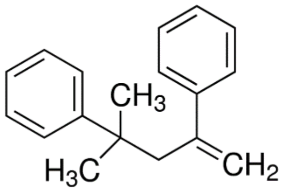
Metal coatings

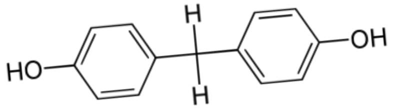
Printing inks

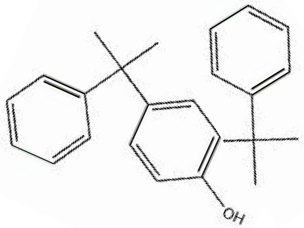
Adhesives

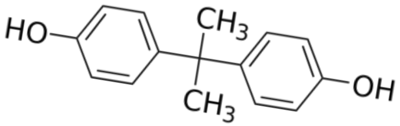


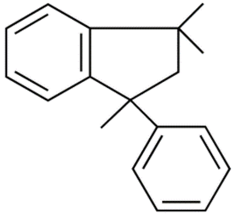
Complex mixtures of hormone active substances in plastics



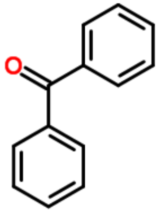


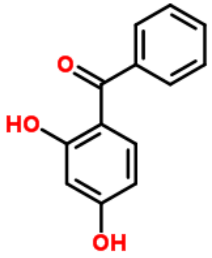


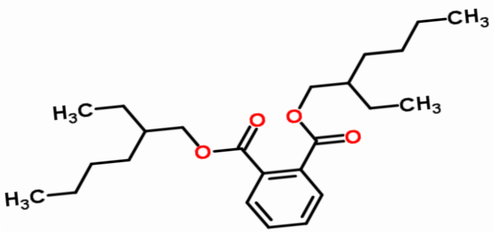


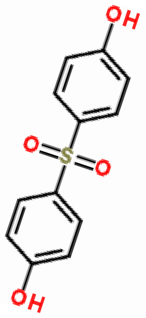


- •Additives (e.g. plasticizers, UV-blockers)
- •By-Products of the polymerization process (e.g. styrene dimers)
- •Monomers (e.g. Bisphenol A, Bisphenol S, phthalates…)
- •Printing color components (e.g. photo initiators)
- •Contaminants
- •….









Known vs Unknown Chemicals - Urgent steps needed…

Muncke et al. Environmental Health (2020) 19:25
https://doi.org/10.1186/s12940-020-0572-5

Environmental Health

COMMENTARY Open Access

Impacts of food contact chemicals on human health: a consensus statement

Check for updates

Jane Muncke[1*], Anna-Maria Andersson[2], Thomas Backhaus[3], Justin M. Boucher[4], Bethanie Carney Almroth[3], Arturo Castillo Castillo[5], Jonathan Chevrier[6], Barbara A. Demeneix[7], Jorge A. Emmanuel[8], Jean-Baptiste Fini[7], David Gee[9], Birgit Geueke[1], Ksenia Groh[1], Jerrold J. Heindel[10], Jane Houlihan[11], Christopher D. Kassotis[12], Carol F. Kwiatkowski[13], Lisa Y. Lefferts[14], Maricel V. Maffini[15], Olwenn V. Martin[16], John Peterson Myers[17,18], Angel Nadal[19], Cristina Nerin[20], Katherine E. Pelch[13], Seth Rojello Fernández[21], Robert M. Sargis[22], Ana M. Soto[23], Leonardo Trasande[24], Laura N. Vandenberg[25], Martin Wagner[26], Changqing Wu[27], R. Thomas Zoeller[28] and Martin Scheringer[4,29]

- **Toxicity** and exposure information is available **only for few of the IAS/NIAS**
- Risk assessment **of unknown chemicals is not possible** under the current regulatory approach
- **Modernize tiered approach for screening** and prioritization
- Addressing **mixture toxicity**
- Modernizing risk assessment by including **endocrine disruption**

OECD Strategy for Endocrine Disrupting Chemicals & EATS CALUX bioassays

OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals

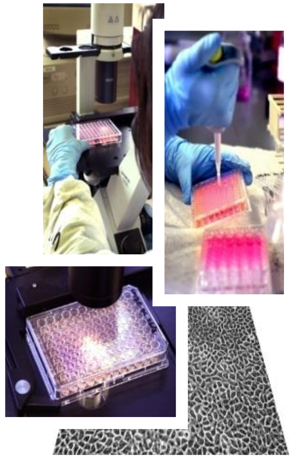
Note: Document prepared by the Secretariat of the Test Guidelines Programme based on the agreement reached at the 6th Meeting of the EDTA Task Force

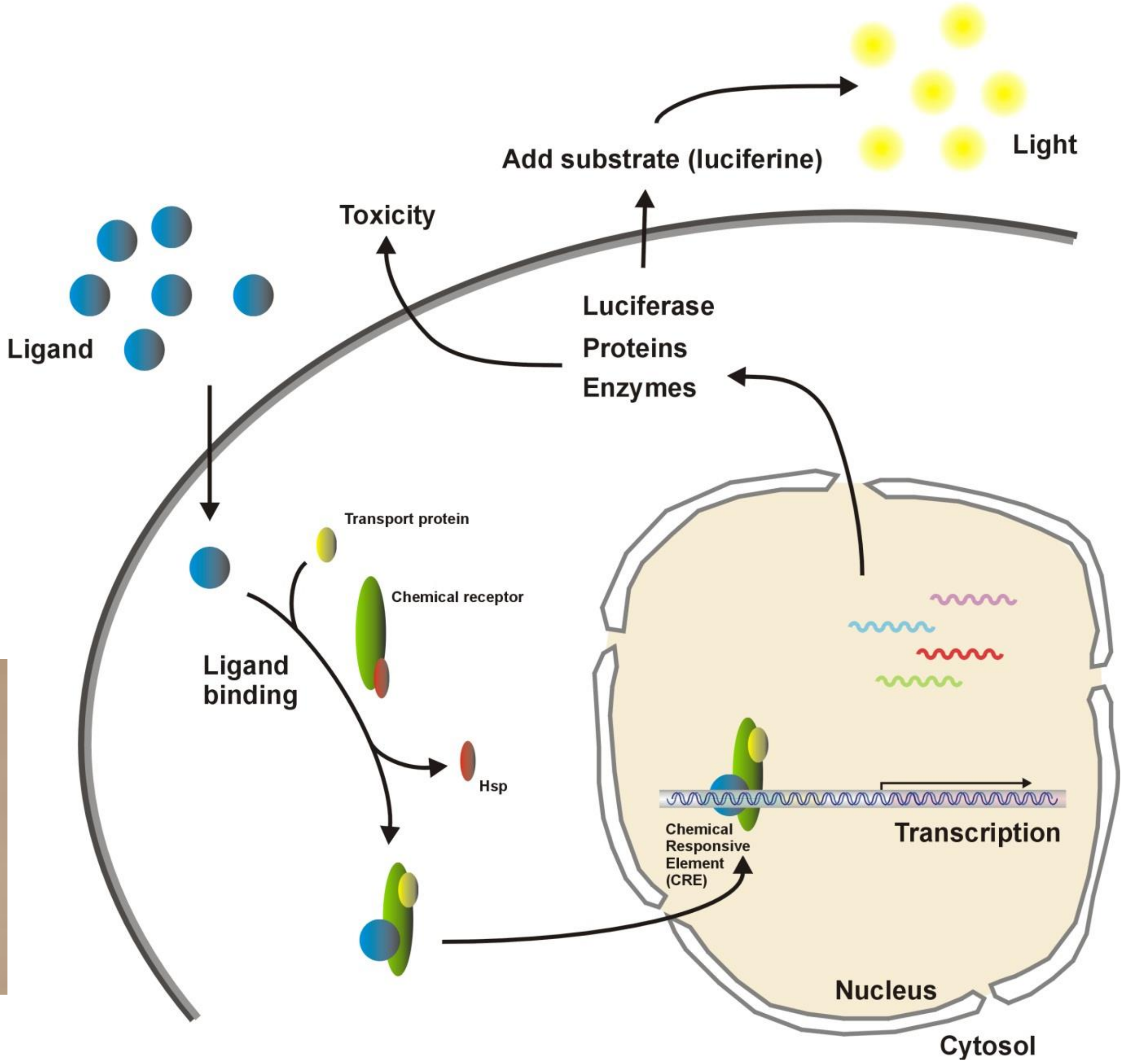
Level 1 Sorting & prioritization based upon existing information	•Physical & chemical properties, e.g., MW, reactivity, volatility, biodegrad •Human & environmental exposure, e.g., production volume, release, use •Hazard, e.g., available toxicological data	
Level 2 *In vitro* assays providing mechanistic data	•ER, AR, TR receptor binding affinity •Transcriptional activation •Aromatase & Steroidogenesis *in vitro* •Aryl hydrocarbon receptor recognition/binding	•High Through Put Pres •Thyroid function •Fish hepatocyte VTG a •QSARs; Others (as app
Level 3 *In vivo* assays providing data about single endocrine Mechanisms and effects	•Uterotrophic Assay (estrogenic related) •Hershberger Assay (androgenic related) •Non-receptor mediated hormone function	•Fish VTG assay (estrog related) •Others (e.g. thyroid)
Level 4 *In vivo* assays providing data about multiple endocrine mechanisms and effects	•Enhanced OECD 407 (endpoints based on endocrine mechanisms) •Male and female pubertal assays •Adult intact male assay	•Fish gonadal histopathology assay •Frog metamorphosis assay
Level 5 *In vivo* assays providing data on effects from endocrine & other mechanisms	•1-generation assay (TG415 enhanced) •2-generation assay (TG416 enhanced) •Reproductive screening (TG421 enhanced) •Combined 28 day/reproduction screening test (TG 422 enhanced)	•Partial and full life cycle assays in fish, birds, amphibians & invertebrates (development & reproduction)

Level 2
In vitro assays providing mechanistic data
•ER, AR, TR receptor binding affinity
•Transcriptional activation
•Aromatase & Steroidogenesis *in vitro*
•Aryl hydrocarbon receptor recognition/binding
•High Through P
•Thyroid functio
•Fish hepatocyt
•QSARs; Others

ER: (anti)Estrogens: ERalpha CALUX (OECD TG455)
AR: (anti)Androgens: AR CALUX (OECD TG458)
TR: Thyroid interference: TR and TTR CALUX (TG in preparation)
S: H295R steroidogenesis (OECD 456)

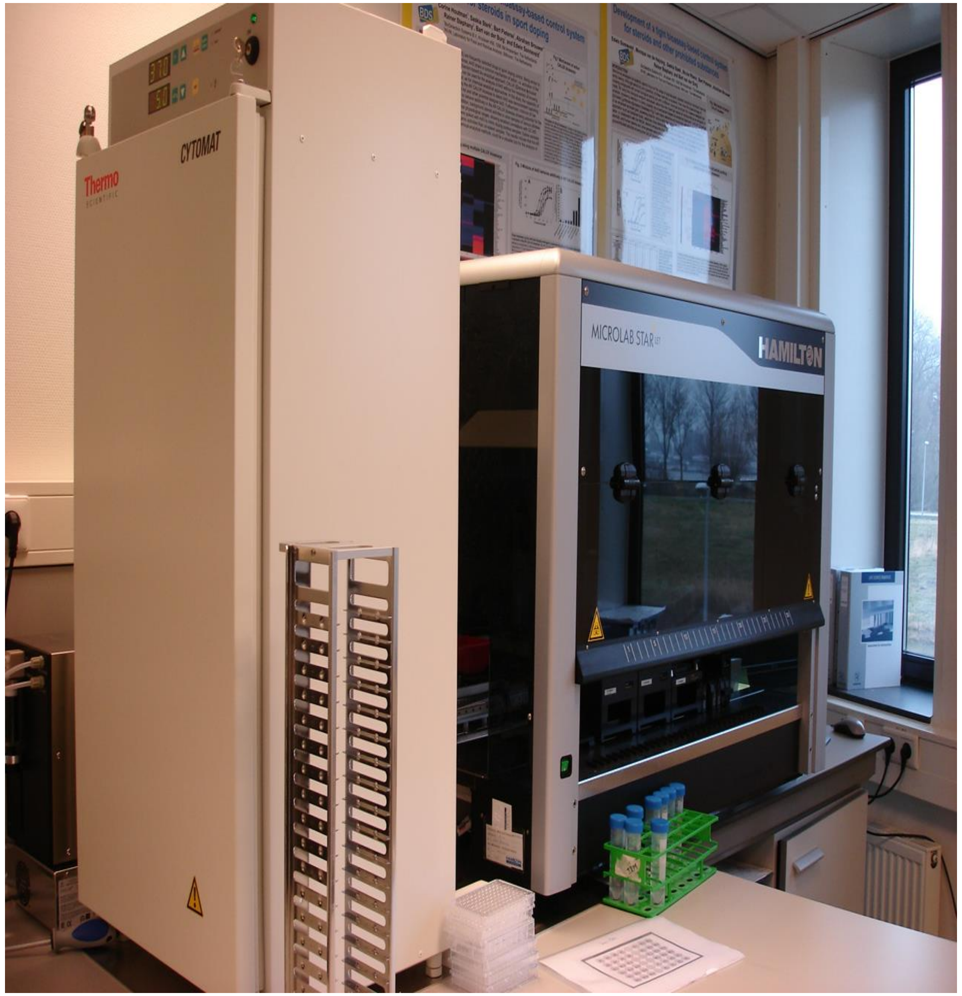
Bioanalysis CALUX for toxic effects for all kinds of pollutants





9

Full automated CALUX cell & sampling handling



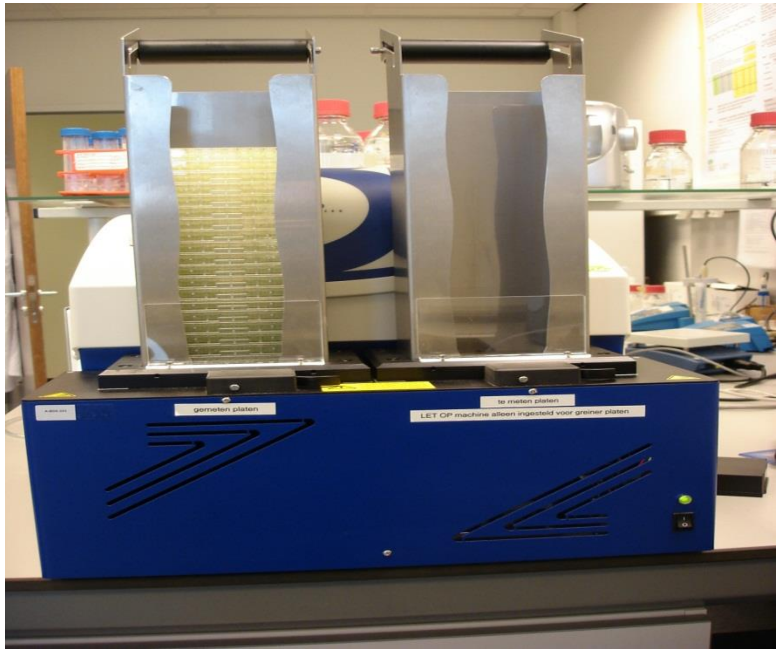
40 samples/hrs

for

a) Seeding cells in 96 well plates and

b) Pipette samples

Full automated Luciferase analysis via Luminometer combined with autosampler



<u>Bioanalysis CALUX:</u>

40 samples/hr for the quantitative analysis of the total amount of pollutant (e.g. hormones, PFAS, dioxins, PAHs) in the sample

The real toxicity/mode of action of the Dirty Dozen POPs & others

- no activity
- EC10 = 1E-3M
- EC10 = 1E-7M

Group	compound	Cytotox10%	Cytotox50%	ERa	ERa-anti	ERb	ERb-anti	AR	AR-anti	PR	PR-anti	GR	GR-anti	TRb	RAR	LXR	PPARa	PPARg	DR	PAH	Hif1a	TCF	AP1	ESRE	NFkB	Nrf2	p21	p53
Dirty Dozen POPs	Chlordane	-5.5	-5	-6.9					-6.5		-6.5		-6													-5		
	DDT	-4.5	-4.2	-6.5		-5.8			-7		-6		-5.5				-5						-4.7	-4.5				
	Dieldrin	-3		-5.8					-7		-7		-5				-5.5											
	Endrin			-5.5					-7		-7																	
	Heptachlor	-5	-4.5	-7.2					-7		-6																	
	Hexachlorobenzene			-6.5					-6		-6								-5									
	Mirex																		-4.2									
	Toxaphene	-5	-4.8	-5.5		-5.5			-6.5		-6.5		-5.5													-6.1		
	PCB118	-4.5							-7		-6.5								-7.1	-6.5			-4.1					
	PCB126	-4.8	-4.4						-6.5		-6						-5		<-10.5	-10.2				-4.5				
	PCB128								-7		-6.5																	
	PCB156	-4.5	-4	-6					-6		-6								-7	-6.8			-4					
	TCDD																		-11.5	-12								
	Furan																			-3.4						-3		
Additional POPs	dibenzo[a,h]anthracene	-4				-7.5												-5	-8	-10								
	dibenzo[a,h]pyrene			-7															-8.9	-9.1			-6.2	-5.5		-6	-6	
	benzo[a]pyrene	-3		-6		-3.9			-6.5		-6								-8.1	-9.7				-4			-4.6	-4.5
	tributyltinacetate	-7	-6.5			-7												-8.5					-6.5	-7		-6.5		
	methylmercury(II)chloride	-5.8	-5.6			-6.4						-6.0									-6.3		-6.3	-6		-6.6	-5.6	-5.7
Heavy metals	Lead chloride	-3.5	-3																					-3.2				
	Mercuric chloride	-4.9	-4.8																				-5.3			-5.5		
	Cadmium chloride	-4.9	-4.7																				-5			-5.3		
	Cobaltous chloride	-3.9	-3.4																-4.2		-4.2		-3.8			-4.5		
	Copper chloride																									-3		
	copper sulfate	-3.4	-3.2																-3.5		-4.5					-5		
	Zinc sulphate	-4.3	-4.1																				-4.1					
	Sodium arsenite	-5.4	-5.2						-6.3		-6.1		-6													-6.5		-4.9
	Nickel(II)chloride	-3.5	-3																		-3.4		-3.1			-4.2		
	chromium(vi)oxide	-5	-4.7																									-4.7

Dirty Dozen POPs: endrocine activity, dioxin receptor (dioxins/PAHs)

Additional POPs: dioxin receptor (PAHs), stress pathways

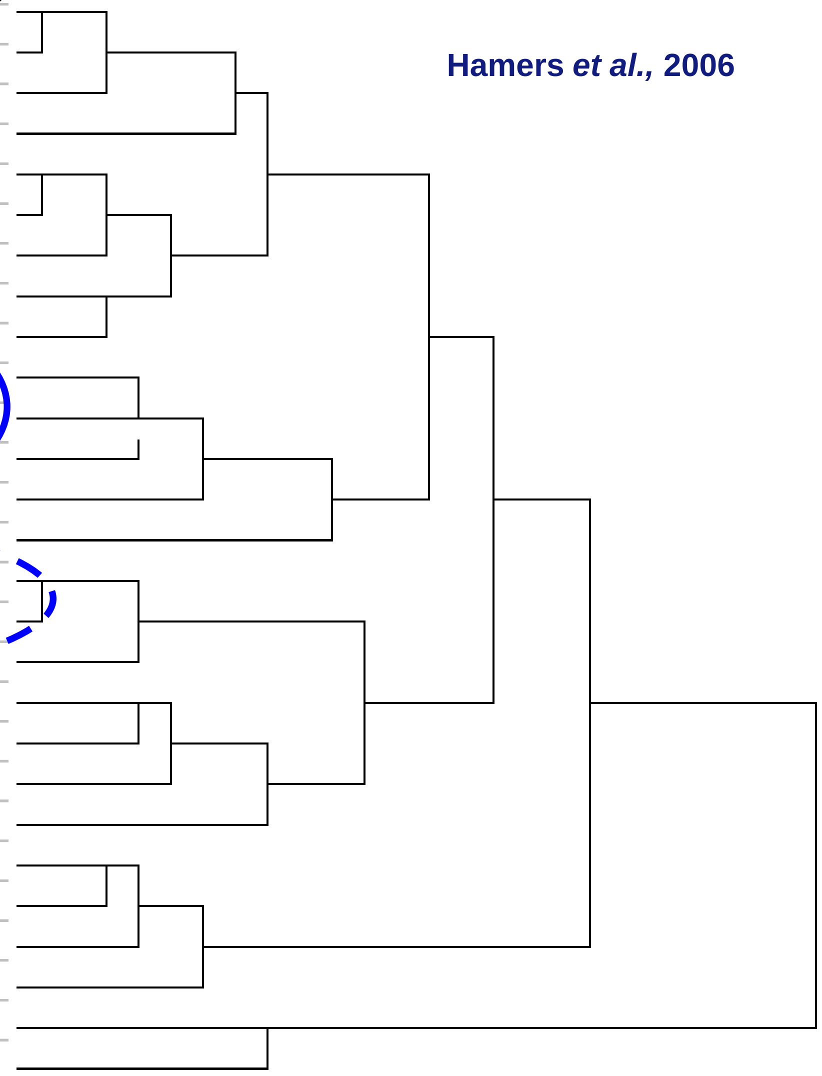
Heavy metals: acute toxicity, stress pathways

In vitro toxicity CALUX profiling

Compound	PR ago	AR ago	TScreen anta	TTR	E2SULT	ER ago	DR anta	ER anta	DR ago	TScreen ago	PR anta	AR anta
BDE39	1	1	1	1	1	1	2	1	1	1	2	3
BDE99	1	1	1	1	1	1	2	1	2	1	2	3
BDE127	1	1	1	2	2	1	3	1	1	2	2	3
BDE185	1	1	1	3	1	1	1	2	1	1	3	2
HBCDD TM	1	1	1	1	1	1	2	2	1	3	3	2
HBCDD beta	1	1	1	2	1	1	2	2	1	3	2	2
HBCDD gamma	1	1	1	1	1	1	2	3	1	4	3	3
BDE28	1	1	1	1	2	2	3	1	1	4	2	3
HBCDD alpha	1	1	1	2	1	1	3	1	1	4	3	3
BDE209	1	1	1	1	2	1	1	1	1	1	1	1
TBBPA-DBPE	1	1	1	3	3	1	1	1	1	1	1	1
BDE169	1	1	1	2	2	1	3	1	1	1	1	1
BDE206	1	1	3	1	3	1	1	1	1	1	1	1
BDE47	1	1	1	2	4	2	3	1	1	1	2	3
BDE190	1	1	1	3	3	1	1	3	3	1	3	3
6OH-BDE47	1	1	1	4	3	1	1	4	3	1	3	3
BDE181	1	1	1	3	1	1	1	3	3	1	3	3
BDE79	1	1	1	1	1	2	1	2	3	1	2	3
BDE153	1	1	1	1	1	1	1	1	4	1	3	2
BDE38	1	1	1	2	1	3	1	1	4	2	2	3
BDE183	1	1	1	1	2	1	1	3	3	3	3	2
BDE19	1	1	1	1	3	3	1	1	1	3	4	5
BDE100	1	1	1	1	2	3	2	1	1	4	3	5
BDE155	1	1	1	1	2	3	1	1	1	3	3	3
BDE49	1	1	1	2	3	3	2	1	2	5	3	4
TBBPA	1	1	1	5	5	1	1	1	1	3	1	1
246-TBP	1	1	1	5	4	1	1	3	1	1	2	2

Hierarchical Clustering

Hamers *et al.*, 2006



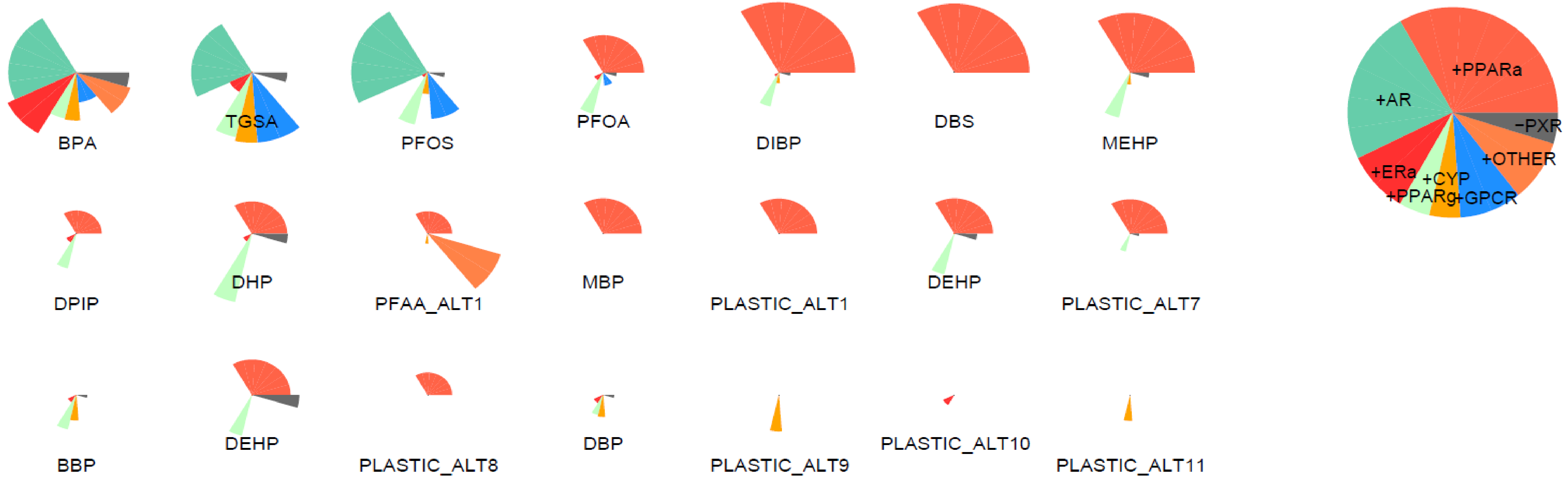
ISO 19040-3: Hormone-like activities of plastic additives by ER CALUX

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]		

ReproTox Predictions for Conventional and Alternative Plasticizers (ToxCast 2011)



Biol Reprod. 2011 Aug;85(2):327-39. Martin MT, Knudsen TB, Reif DM, Houck KA, Judson RS, Kavlock RJ, Dix DJ.

CALUX toxic profile of plastic additives

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

activity - +

FDCA - little or no endocrine effects

- biobased building block to replace terephthalate in PET

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	-	-	-	-	-	-	-	-	-	-	-	-	-

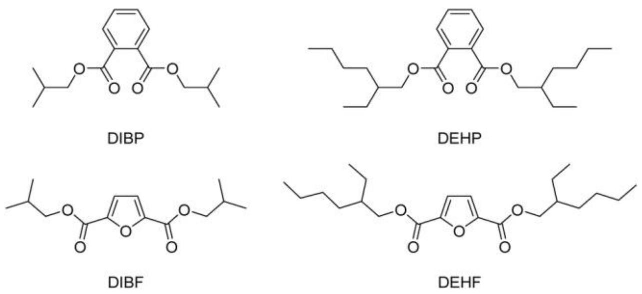
activity

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

In vitro toxicity of Bioplastics and Plant-based materials (I)
(Zimmermann et al 2020)

Environment International 145 (2020) 106066

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Environment International

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

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]

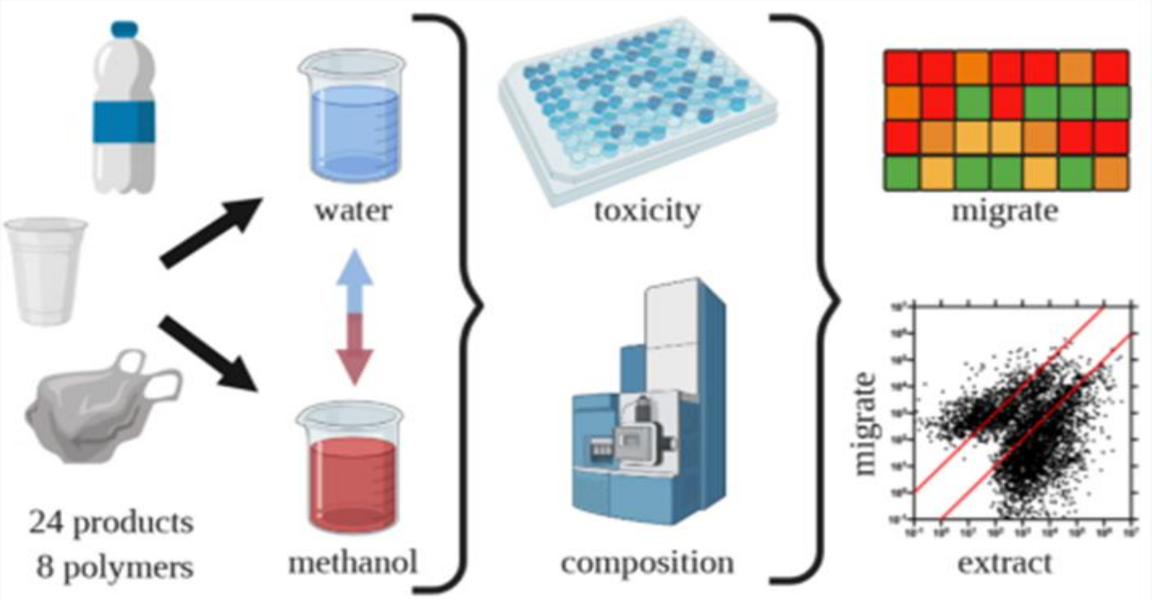


Table 1
Bioplastics and plant-based materials analyzed in this study and total number of chemicals features detected by UPLC-QTOF-MS/MS. FCM: Indication that material is suitable for food contact, Type: Raw material (RM), final product (P).

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

In vitro toxicity of Bioplastics and Plant-based materials (II)

(Zimmermann et al 2020)

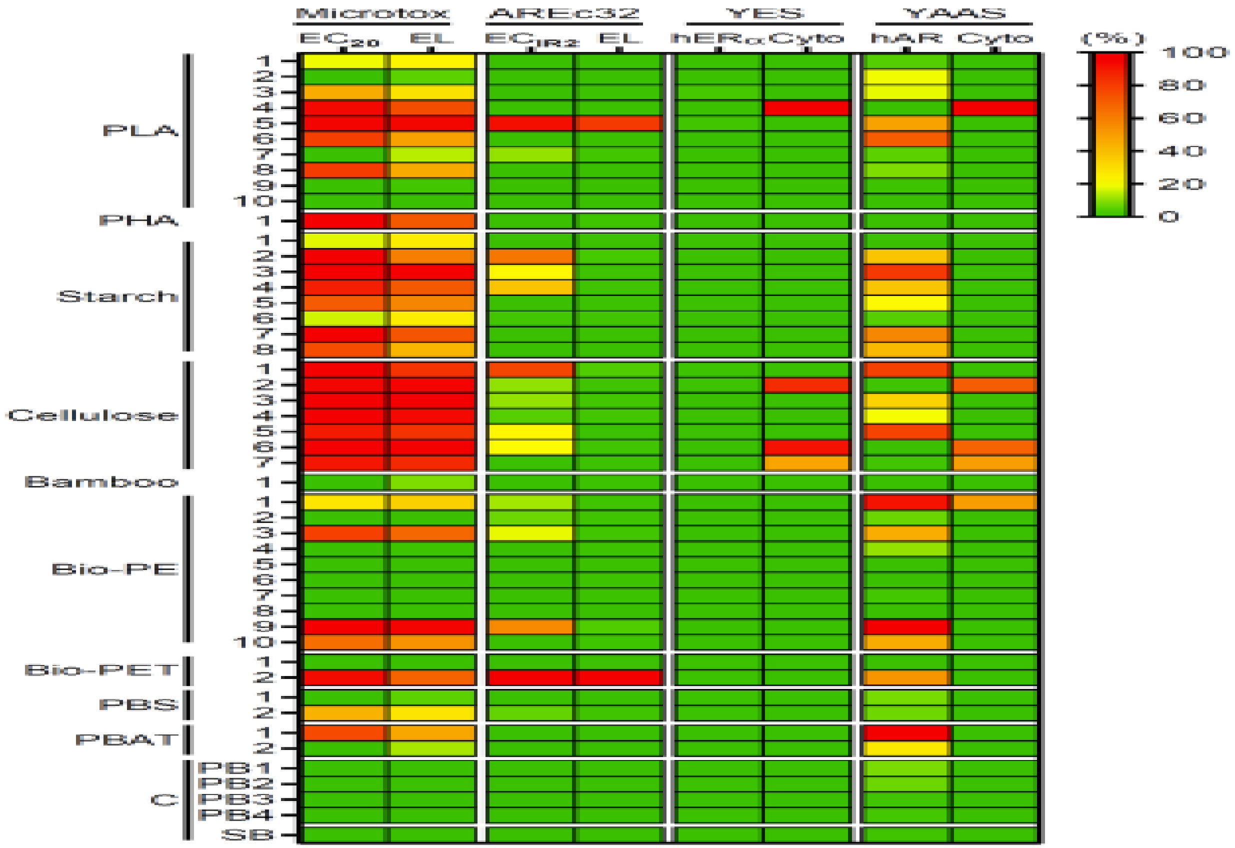


Fig. 1. Toxicological signature of bioplastics and plant-based materials based on baseline toxicity (Microtox), oxidative stress response (AREc32) as well as estrogenic (YES) and antiandrogenic activities (YAAS). The results are presented as effect concentrations (EC_{20}, EC_{IR2}), effect levels (EL), relative receptor activation/inhibition and EC_{20} for cytotoxicity (Cyto). Results are presented as gradient from 0 (green) to 100% (red). The endocrine activities were used as such v sults were normalized to the lowest and highest effect obser ctive endpoint. For AREc32 ELs, the highest non-cytotoxic ab. S4) were used. (For interpretation of the references to col legend, the reader is referred to the web version of this article

- 43 everyday bio-based products were tested by in vitro bioassays:

- 67% of the samples induced **baseline toxicity** (Microtox)
- 42% **oxidative stress** (AREc32),
- 23% **anti-androgenicity** (anti-YAAS) and
- Several sample **cytotoxicity** and **estrogenicity** (YES)

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

In vitro toxicity of Bioplastics and Plant-based materials (III)

(Zimmermann et al 2020)

- 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 (I)

(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	+

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

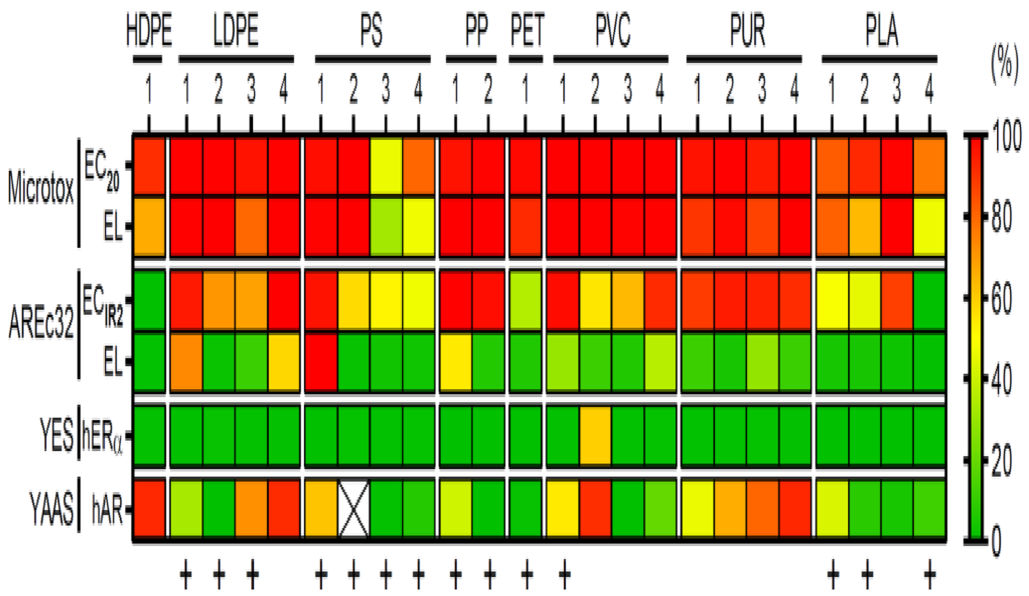


Figure 1. *In vitro* toxicity of chemicals migrating from plastic consumer products. Baseline toxicity (Microtox) and oxidative stress response (AREc32) are presented as effect concentrations inducing 20% baseline toxicity (EC_{20}) or an induction ratio of 2 (EC_{IR2}) as well as effect levels (EL) at the highest analyzed noncytotoxic concentration. Estrogenic (YES) and antiandrogenic activities (YAAS) are shown as relative (%) activation of the human estrogen receptor α ($hER\alpha$) and inhibition of the androgen receptor (hAR). Note: x, all analyzed concentrations were cytotoxic; +, food contact materials.

- Toxicological and chemical profiles leaching into water from 24 everyday plastic products covering eight polymer types.
-
- **Migration experiments over 10 days at 40 °C** and analyzed the migrates using four in vitro bioassays.
- All migrates induced **baseline toxicity** (Microtox)
- 22 oxidative stress response (AREc32)
- 13 **antiandrogenicity** (anti-YAAS), and
- **1 estrogenicity** (YES).

Zimmermann et al (2021). Environ. Sci. Technol. 55, 11814ff

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

- Overall, **between 17 and 8681 relevant chemical features** were present in the migrates.
- **Between 1 and 88% of the plastic chemicals associated with one product** were migrating.
- Only ~8% of all detected features were identified = implying that **most plastic chemicals remain unknown.**
- **LDPE, PVC, and PU induced most toxicological endpoints**
- These results demonstrate that plastic products readily **leach many more chemicals than previously known**, some of which are toxic in vitro.
- This highlights that **humans are exposed** to many more plastic chemicals than currently considered in public health science and policies.

Zimmermann et al (2021). Environ. Sci. Technol. 55, 11814ff

Latest study about In vitro toxicity profiling of Plastic Consumer Products
(Stevens et al 2022)

- **39 plastic food contact materials** from Germany, Norway, South Korea the UK, and the US, covering the seven polymer types with the highest global market share were extracted and analyzed with **CALUX reporter gene assays: Pregnane X receptor (PXR), peroxisome proliferator receptor gamma (PPARgamma), estrogen receptors alpha (ER) and androgen receptor (AR).**
- The chemicals extracted from **36 out of 39 plastics activated or inhibited one or more receptors with activities up to 100% of** the respective **positive controls**.
- The **PXR was activated by 36, the ERalpha by 25, the PPARgamma by 23 samples, while the AR was inhibited by 14 of 39 samples.**

- In total, **> 16,000 unique chemical features** with the number of features per sample ranging from 37 to 9,936 were detected.
- Only **16% of the chemical** features were tentatively **identified** using spectral libraries and *in silico* tools.

- These results confirm that many plastic materials contain **endocrine disrupting chemicals**, which needs to be identyfied.
- This study highlights again the importance of analyzing **whole mixtures of finished products** and contributes to improving our understanding of plastics as a source of exposure to endocrine disrupting chemicals
- These results demonstrate that plastic products readily **leach many more chemicals than previously known**, some of which are toxic in vitro.

Stevens et al (2022). Abstract SETAC-EU Conference

How to make safer & greener plastic & bioplastic devices: Industrial innovation

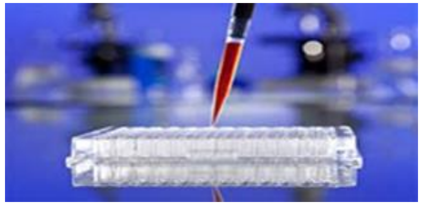

2017

R&D recommendations for the Chemical and Biological Migration Screening of metal packaging materials

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

Migration or full material testing

Bioassays battery



CHEMISTRY: LC-MS DATA

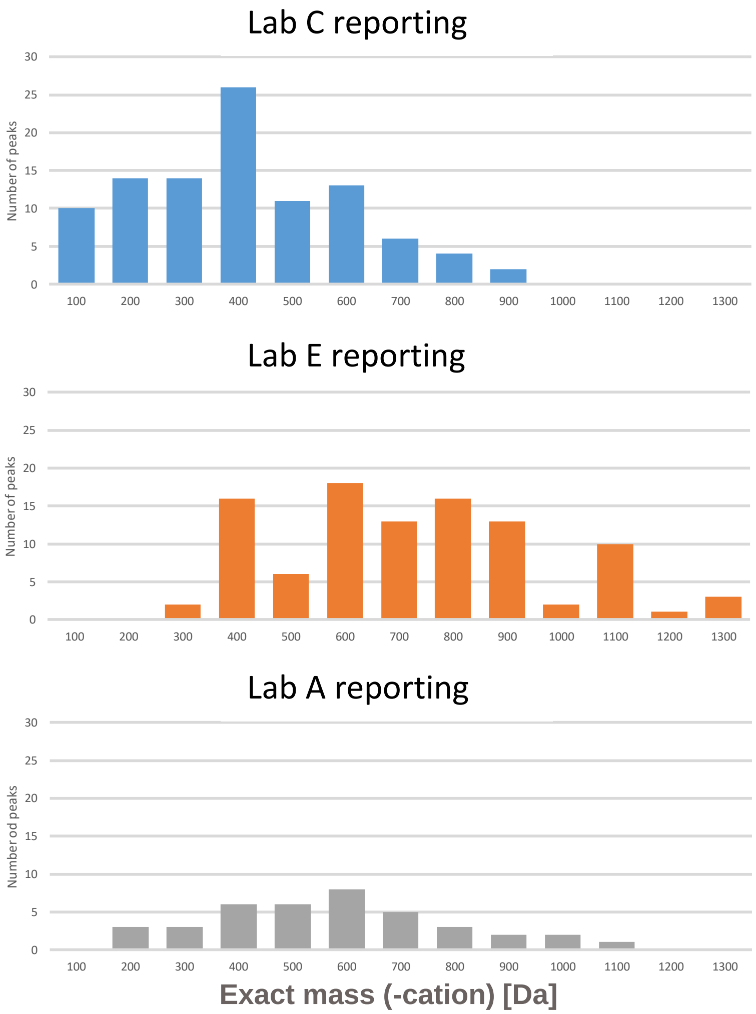
DATA TOP 100 PEAKS THREE LABS-DATA DESCRIPTION

Lab C
100 peaks
89 distinct peaks*

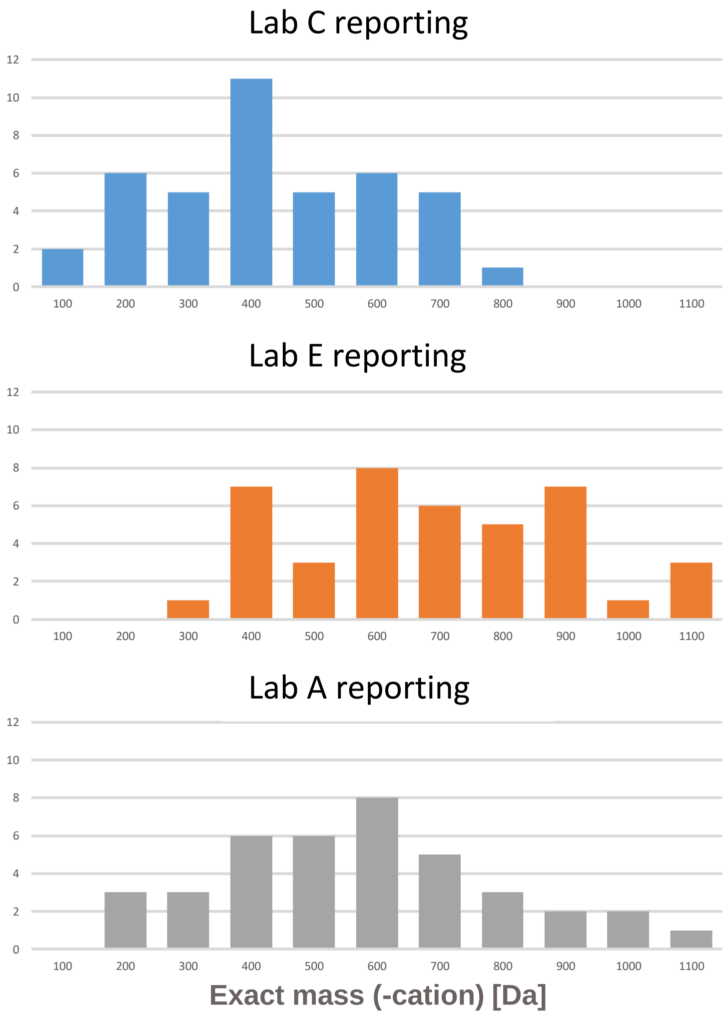
Lab E
109 peaks
72 distinct peaks*

Lab A
41 peaks
38 distinct peaks*

*Distinct peaks: Difference on exact mass (-cation) > 0.05 Da



Distribution top 100 peaks



Distribution top 41 peaks

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

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

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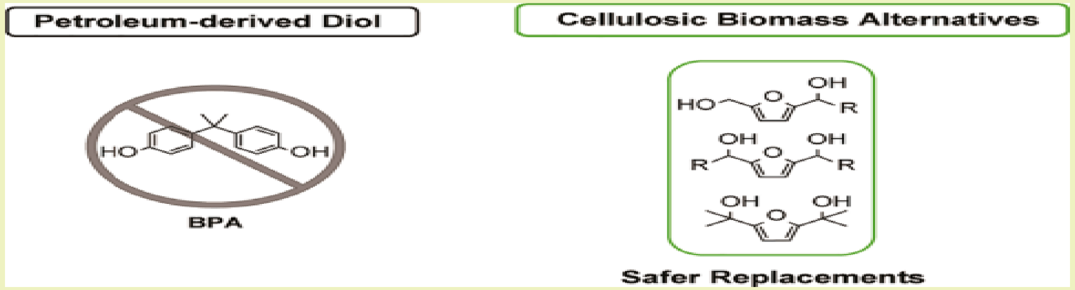
ABSTRACT: A series of asymmetric and symmetric diols were prepared in high yields from biomass-derived feedstocks 5-hydroxymethyl furfural (HMF) and 2,5-diformyl furan (DFF) as potential replacements for bisphenol A (BPA). The diols were screened for estrogenic, androgenic, antiandrogenic, and antithyroid 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

PFAS in disposable food packaging and tableware: Thyroid hormone transport interferences by TTR-FITC-T4 bioassay

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

Toys & consumer products with high levels of brominated dioxins (Arnika report 2018)

Country / Sample	Type	PBDD/Fs (pg TEQ/g)[1]	DR CALUX (pg TEQ/g)	PBDEs (ug/g)[2]	HBCD (ug/g)	TBBPA (ug/g)
Argentina ARG_04	Rubik's-like cube	727	1,200	708	1	na[3]
Brazil BRZ-T-7A	Toy, car	750	590	169	0.2	8
Cambodia KAM-H-1	Hair diadem	1,950	1,500	358	0.3	10
Canada CA-H-1C	Hair rack	1,500	1,300	718	< 0.01	1
Czechia JI_11	Cube	2,159	17,000	2,614	91	na[3]
Czechia SIX_02	Hairclip	60	210	1,623	8	na[3]
France FR-T-3	Toy revolver	2,058	520	1,077	1	314
Germany D-TO7	Key fob	3,821	820	511	2	307
India IND_11	Rubik's-like cube	690	1,300	593	2	na[3]
Japan JP-O-1	Smart phone holder	1,200	560	693	0.5	37
Nigeria NIG_06	Rubik's-like cube	860	4,800	1,780	9	na[3]
Nigeria NIG_11	Rubik's-like cube	56	370	1,218	8	na[3]
Portugal PT-T-10a	Toy small guitar	1,137	270	3,318	2	37

High throughput extraction of plastic in our study here

A) <u>THF/hexane:</u>

- 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.

<u>B) 50% Ethanol/Water:</u>

- 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.

<u>C) 20% Ethanol/Water:</u>

- 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: Sample N3 - HDPE

Nr.	Polymer type	Sampling location	Sample ID	Sampling date	Pellet origin	Pellet feedstock Material	Polymer characteristics	Used additives	Pellet color	Pellet uses	Sending samples Germany, Spain & Netherlands
N3	High Density Polyethylene (HDPE)	Nigeria, Anambra, Onitsha	01 Nigeria HDPE	02/18/2023	National feedstock	Water tanks	Flexible	Master batch	Black	Jerrycan, grease container, spoons	Neth Ger Spa

Technic	Parameter	Result	Unit
Extraction: THF/hexane			
Cytotox CALUX	Celldeath	41	ug Tributyltin acetate eq./
AR CALUX	Anti-androgens	LOQ (<8.3)	ug Flutamide eq./g
P53 CALUX	P53 transcriptional activators	LOQ (<0.28)	ug Actinomycin D eq./gra
ERa CALUX	Estrogens	LOQ (<0.13)	ng 17b Estradiol eq./g
PAH CALUX	Polycyclic aromatic hydrocarl	2000	ng Benzo[a]pyrene eq./g
Extraction: 50% ethanol			
Cytotox CALUX	Celldeath	0.55	ug Tributyltin acetate eq./
AR CALUX	Anti-androgens	LOQ (<4.0)	ug Flutamide eq./g
P53 CALUX	P53 transcriptional activators	LOQ (<0.027)	ug Actinomycin D eq./gra
ERa CALUX	Estrogens	LOQ (<0.044)	ng 17b Estradiol eq./g
PAH CALUX	Polycyclic aromatic hydrocarl	7000	ng Benzo[a]pyrene eq./g

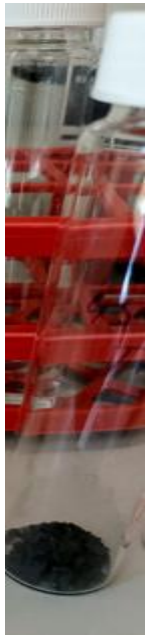
In vitro toxicity profiling by CALUX panel:
Sample N9 - LDPE

Nr.	Polymer type	Sampling location	Sample ID	Sampling date	Pellet origin	Pellet feedstock Material	Polymer characteristics	Used additives	Pellet color	Pellet uses	Sending samples
N9	Low Density Polyethylene (LDPE)	Nigeria, Anambra, Onitsha	04 Nigeria LDPE	45141	National feedstock	Toothbrush, disposable food container (single use), cosmetics containers	Flexible	Master batch	Mixed colour	cover for plates and plastic containers, spoons	Neth Ger Spa

Extraction: THF/hexane			
Cytotox CALUX	Celldeath	32	ug Tributyltin acetate eq./
AR CALUX	Anti-androgens	LOQ (<8.3)	ug Flutamide eq./g
P53 CALUX	P53 transcriptional activators	LOQ (<0.28)	ug Actinomycin D eq./gra
ERa CALUX	Estrogens	LOQ (<0.13)	ng 17b Estradiol eq./g
PAH CALUX	Polycyclic aromatic hydrocarl	610	ng Benzo[a]pyrene eq./g
Extraction: 50% ethanol			
Cytotox CALUX	Celldeath	0.34	ug Tributyltin acetate eq./
AR CALUX	Anti-androgens	14	ug Flutamide eq./g
P53 CALUX	P53 transcriptional activators	LOQ (<0.029)	ug Actinomycin D eq./gra
ERa CALUX	Estrogens	0.47	ng 17b Estradiol eq./g
PAH CALUX	Polycyclic aromatic hydrocarl	600	ng Benzo[a]pyrene eq./g

In vitro toxicity profiling by CALUX panel: Sample N22 - PVC

Nr.	Polymer type	Sampling location	Sample ID	Sampling date	Pellet origin	Pellet feedstock Material	Polymer characteristics	Used additives	Pellet color	Pellet uses	Sending samples Germany, Spain & Netherlands
N22	Polyvinyl Chloride (PVC)	Nigeria, Anambra, Onitsha	05 Nigeria PVC	45110	National feedstock	Sandals or footwear	Semi-rigid	Master batch	Black	Slippers, carpet	Neth Ger Spa

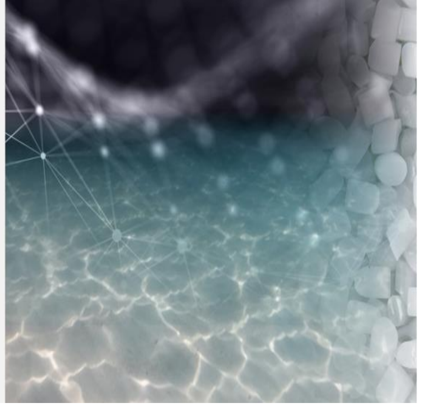


Extraction: THF/hexane			
Cytotox CALUX	Celldeath	1.6	ug Tributyltin acetate eq./
AR CALUX	Anti-androgens	390	ug Flutamide eq./g
P53 CALUX	P53 transcriptional activators	LOQ (<0.11)	ug Actinomycin D eq./gra
ERa CALUX	Estrogens	5.7	ng 17b Estradiol eq./g
PAH CALUX	Polycyclic aromatic hydrocarl	45000	ng Benzo[a]pyrene eq./g
Extraction: 20% ethanol			
Cytotox CALUX	Celldeath	1.1	ug Tributyltin acetate eq./
AR CALUX	Anti-androgens	2.8	ug Flutamide eq./g
P53 CALUX	P53 transcriptional activators	LOQ (<0.096)	ug Actinomycin D eq./gra
ERa CALUX	Estrogens	0.044	ng 17b Estradiol eq./g
PAH CALUX	Polycyclic aromatic hydrocarl	470	ng Benzo[a]pyrene eq./g

In vitro toxicity profiling by CALUX panel: Sample N24 - PP

Nr.	Polymer type	Sampling location	Sample ID	Sampling date	Pellet origin	Pellet feedstock Material	Polymer characteristics	Used additives	Pellet color	Pellet uses	Sending samples Germany, Spain & Netherlands
N24	Polypropylene (PP)	Nigeria, Anambra, Onitsha	02 Nigeria PP	45110	National feedstock	Toys, jars, cup, pellets	Flexible	Master batch	White and pick	Pallets, plastic bottles, beverage cup	Neth Ger Spa

Extraction: THF/hexane			
Cytotox CALUX	Celldeath	16	ug Tributyltin acetate eq./
AR CALUX	Anti-androgens	12	ug Flutamide eq./g
P53 CALUX	P53 transcriptional activators	LOQ (<0.084)	ug Actinomycin D eq./gra
ERa CALUX	Estrogens	LOQ (<0.034)	ng 17b Estradiol eq./g
PAH CALUX	Polycyclic aromatic hydrocarl	1500	ng Benzo[a]pyrene eq./g
Extraction: 50% ethanol			
Cytotox CALUX	Celldeath	LOQ (<0.34)	ug Tributyltin acetate eq./
AR CALUX	Anti-androgens	10	ug Flutamide eq./g
P53 CALUX	P53 transcriptional activators	LOQ (<0.0096)	ug Actinomycin D eq./gra
ERa CALUX	Estrogens	0.16	ng 17b Estradiol eq./g
PAH CALUX	Polycyclic aromatic hydrocarl	3200	ng Benzo[a]pyrene eq./g



Take home message for bioplastic

- 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 bioplastic testing** following industrial leaders…

THANK YOU FOR YOUR ATTENTION

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