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Effect-based safety assessment of bio-based chemicals:
a case study on bio-based plastics

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Introduction

The annual production of plastics is ~300 million tonne, with the vast majority of petrochemical origin. Biobased plastics, building blocks and additives would therefore greatly benefit the biobased economy. Examples of biobased plastics include PLA and PEF. However, since bioplastics are relatively new, not much is known about their safety, especially when used as food contact materials. To assure the safety of these biobased plastics, effect-based assays are perfectly suited because they provide information on the effect of compounds in complex mixtures without the requirement of prior knowledge on their chemical structure.

Methods and Materials

We have recently developed a range of human-cell based reporter gene bioassays that allow effect-based safety assessment of chemicals and chemical mixtures[1]. These assays can be used in a high throughput mode to predict endocrine activity of chemicals, genotoxicity, and several other types of toxicity.

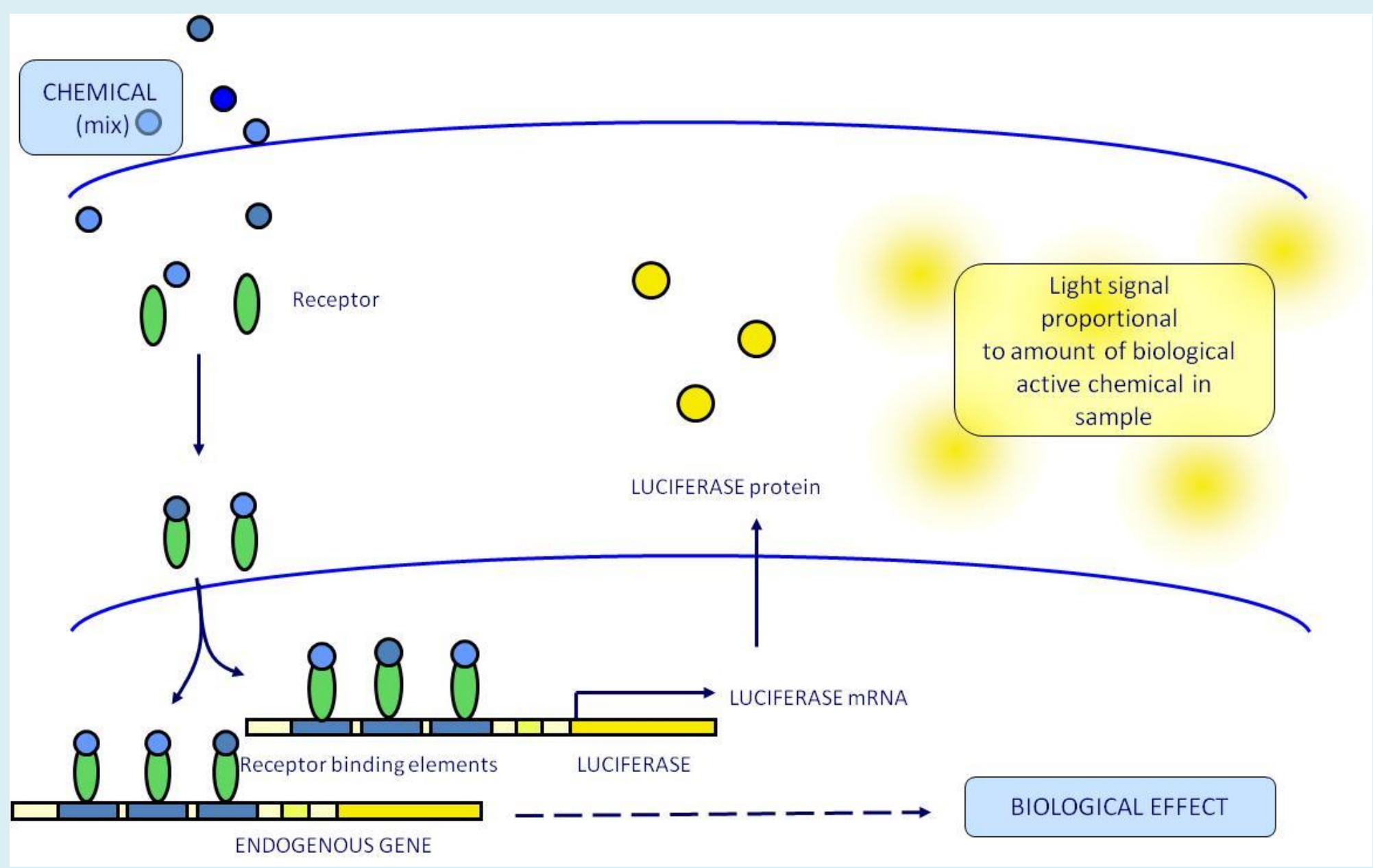
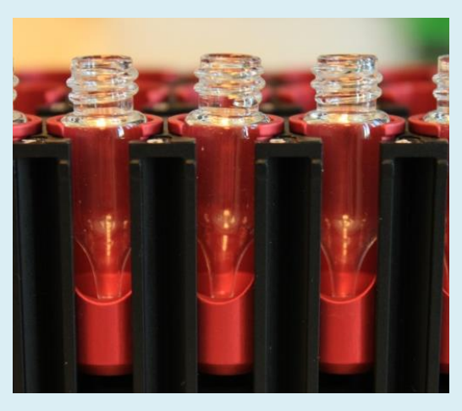


Figure 1: Principle of a reporter-gene assay



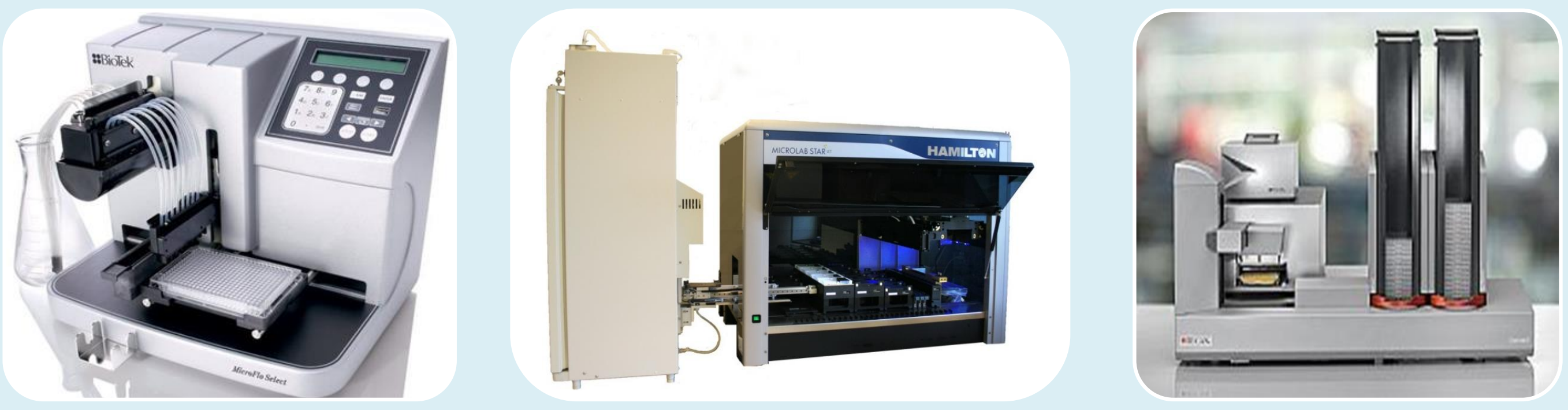
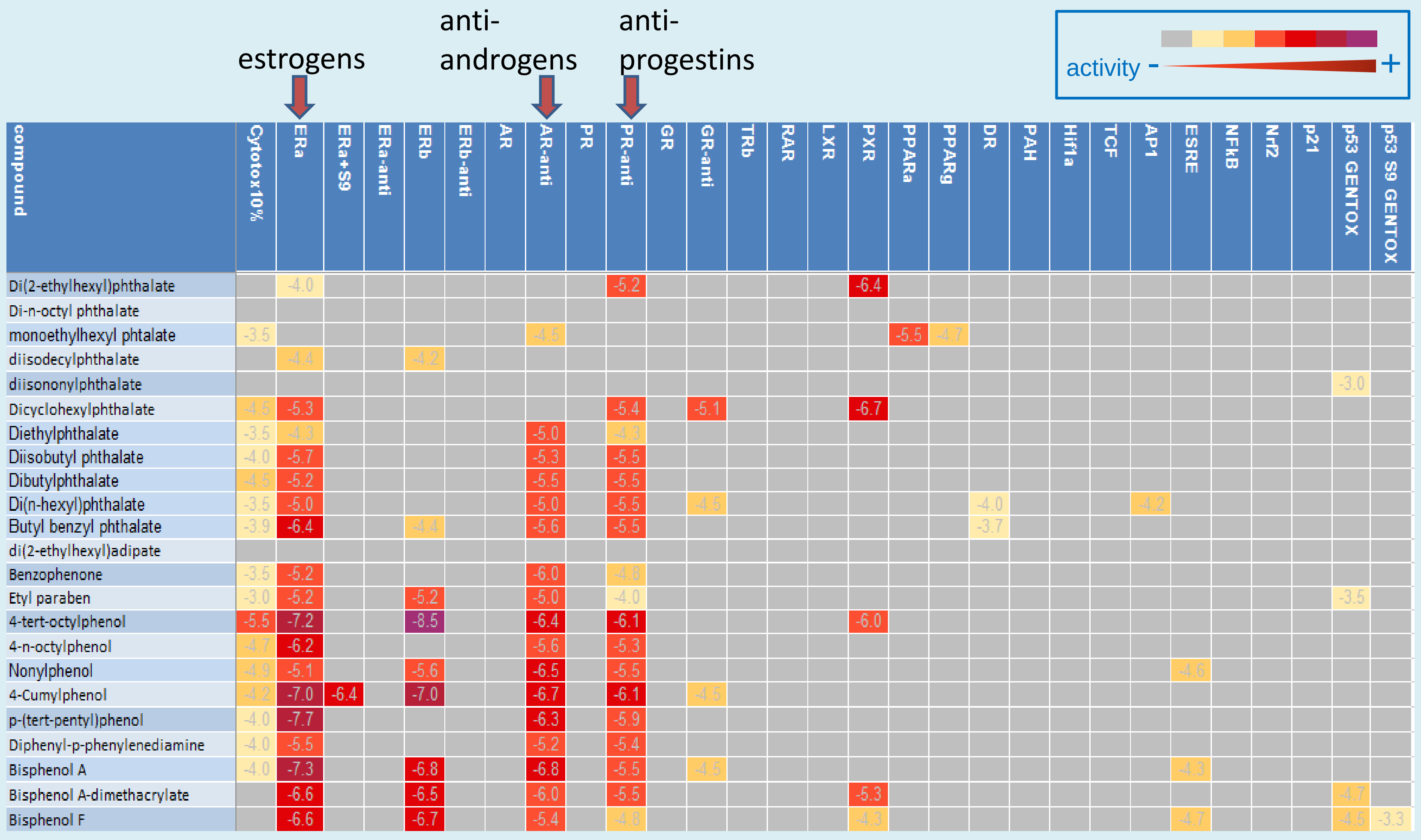


Figure 2: High-throughput Screening equipment

Plastic Additives activity profile

A selection of known plastic components and additives was screened on the bioassay panel (table 1). Most compounds were active on the endocrine assays: they act as estrogens, anti-androgens and anti-progestins. These results are in good agreement with available data on these compounds.



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

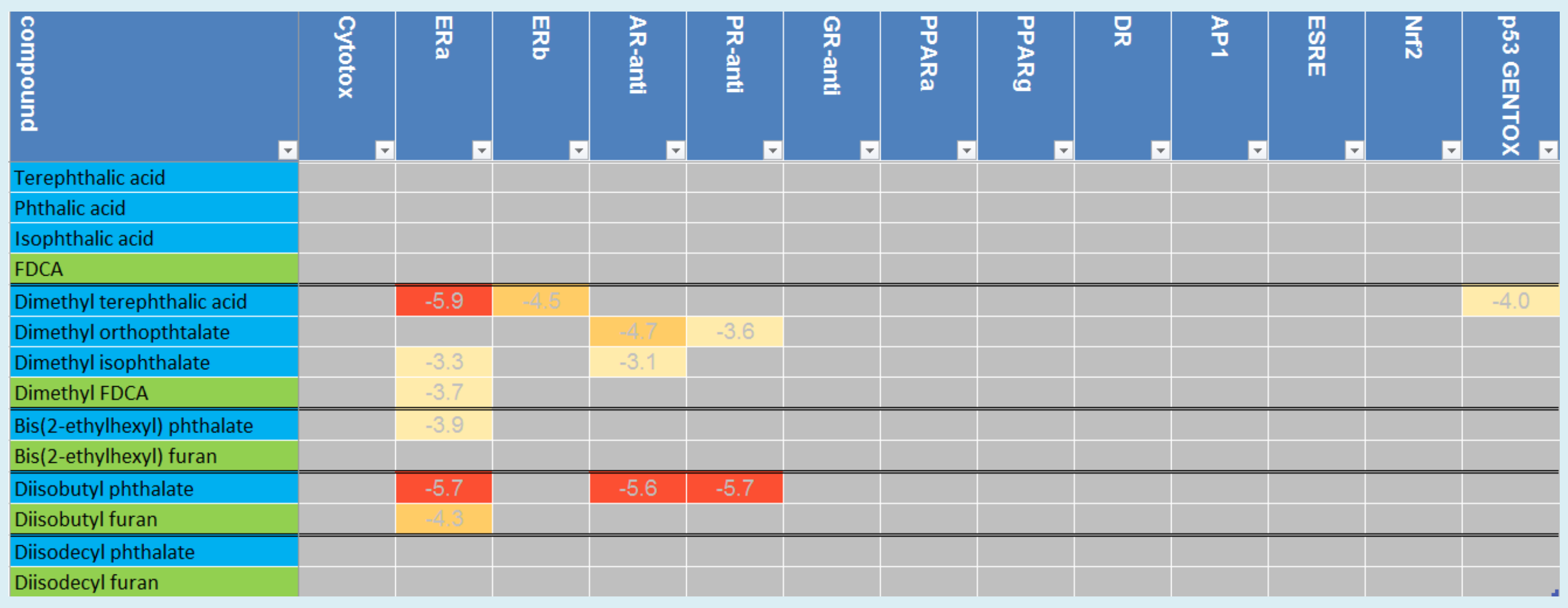
Table 1: activity profile of common plastic components

References

1 Van der Burg et al., In "High throughput screening methods in toxicity testing". John Wiley and Sons, Inc. New York. pp. 519-532, 2013

Phthalate vs Furan

The furan-based Furan-2,5-dicarboxylic acid (FDCA) is a very promising alternative for terephthalic acid in PET bottles. The toxicity profile of furan-based plastic additives was compared to that of their phthalate-based counterparts using the bioassay panel (table 2). The furan-based compounds (green) were active on fewer assays, and showed a lower potency compared to their phthalate-based counterparts (blue).



compound	Cytotox	ERa	ERb	AR-anti	PR-anti	GR-anti	PPARa	PPARg	DR	AP1	ESRE	Nrf2	p53 GENTOX
Terephthalic acid													
Phthalic acid													
Isophthalic acid													
FDCA													
Dimethyl terephthalic acid		-5.9	-4.5										-4.0
Dimethyl orthopthtalate				-4.7	-3.6								
Dimethyl isophthalate		-3.3		-3.1									
Dimethyl FDCA		-3.7											
Bis(2-ethylhexyl) phthalate		-3.9											
Bis(2-ethylhexyl) furan													
Diisobutyl phthalate		-5.7		-5.6	-5.7								
Diisobutyl furan		-4.3											
Diisodecyl phthalate													
Diisodecyl furan													

Table 2: phthalate-based versus furan-based plastic additives

activity - +

Plastic Migration

A plastic migration experiment was carried out according to regulations EC 10/2011 on different plastics. The leachates were analysed for estrogenic activity on the ERα CALUX. Only polyethylene and brightly coloured, flexible PET were positive.

Material	Special remarks	Estrogen Equivalents (mg/kg food simulant)
PET	Hard, food grade	<LOD
PET	BPA/phthalate-free; pink	5.1E-8
PET	BPA/phthalate-free; blue	8.2E-9
PET	Microwave/freezer-safe	<LOD
HD-polyethylene	Suitable as FCM	7.3E-7
LD-polyethylene	Suitable as FCM	1.8E-6
PVC	Non-food grade	<LOD
Polypropylene	Hot use	<LOD
Polypropylene	Cold use	<LOD
PolyLacticAcid	BioBased	<LOD

Table 3: estrogenic activity reported in estrogen equivalents (EEQs).
LOD: limit of detection

Metabolism

To further improve the predictive capacity of the assays, current research now focuses on a methodology to include phase I and phase II metabolic steps in the assays to mimic in vivo hepatic metabolism. For example, methoxychlor is converted by liver S9 mix into a more estrogenic metabolite, whereas bisphenol A is unaffected by phase I metabolism, but inactivated by phase II metabolism.

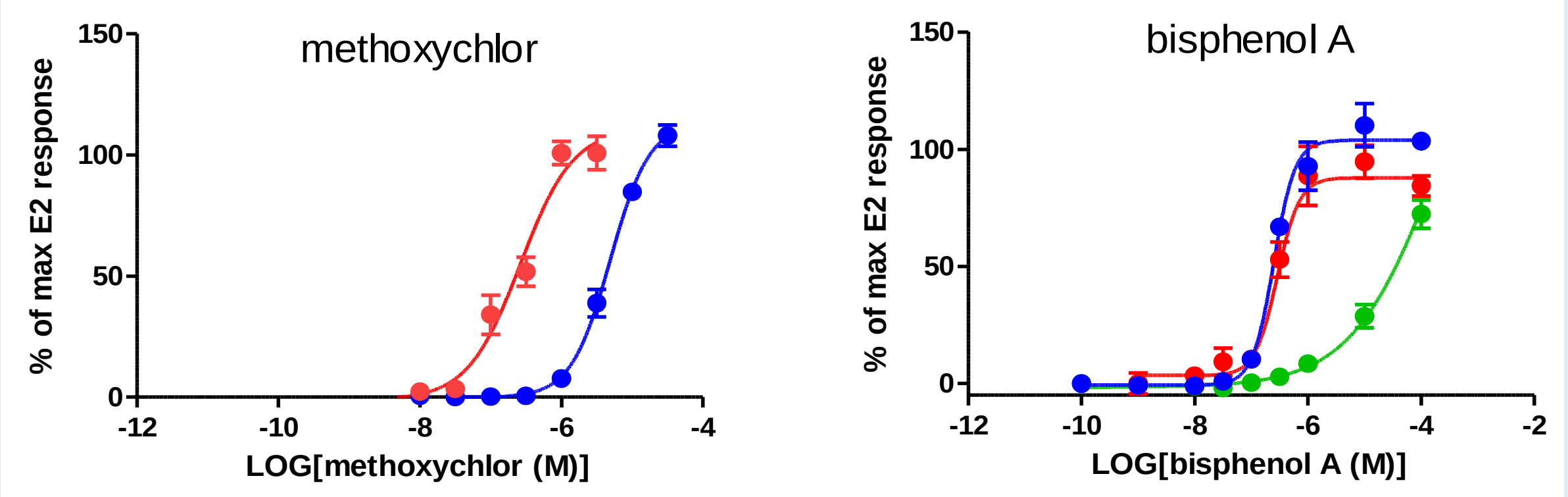


Figure 3: methoxychlor and BPA on the ERα CALUX.
Blue: no liver S9;
Red: phase I enzymes; Green: phase I&II enzymes.

Conclusions

The panel of human-cell based reporter gene bioassays is a useful tool to evaluate the safety of packaging materials and plastic additives. These hazard profiles can be the basis to rapidly select the most promising candidates.

In a broader sense, since the bioassays can analyse complex mixtures, they can be used to monitor every step of any biobased production process: from biomass via waste streams and intermediates to the final product.