Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.
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Electronic Supplementary Information: Solid-phase extraction as sample preparation of water samples for cell-based and other *in vitro* bioassays

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Table S1: Studies that have determined the $BEQ_{bio,extract}/BEQ_{chem,extract}$ ratio to assess chemical SPE recovery expressed as effect in a water matrix.

Study	Extraction Sorbent	Water Matrix	Spiked Chemical Mixture	Bioassay	Endpoint	$BEQ_{bio,extract}/BEQ_{chem,extract}$
Leusch *et al.*[5]	Oasis HLB	Ground water, raw wastewater, treated wastewater, river water	Eight estrogenic compounds (17β-estradiol, estrone, estriol,17α-ethinylestradiol, 4-t-octylphenol, 4-nonylphenol, bisphenol A, benzyl butyl phthalate)	YES	Activation of ER	0.3-0.79
				ER CALUX	Activation of ER	0.98
				MELN	Activation of ER	0.46
				KBluc	Activation of ER	1.64
				E-SCREEN	Cell proliferation	0.68-0.97
Kolkman *et al.*[6]	Oasis MCX	Surface Water	39 chemicals, including hormones, pesticides, pharmaceuticals and industrial compounds	ER CALUX	Activation of ER	0.44 (BEQ_{bio} 2.2 ng/L; BEQ_{chem} 5 ng/L)
				AR CALUX	Activation of AR	0.05 (BEQ_{bio} 8.2 ng/L; BEQ_{chem} 177 ng/L)
				GR CALUX	Activation of GR	1.06 (BEQ_{bio} 110 ng/L; BEQ_{chem} 104 ng/L)
				PR CALUX	Activation of PR	0.02 (BEQ_{bio} 0.91 ng/L; BEQ_{chem} 53 ng/L)
				TRβ CALUX	Activation of TRβ	0.38 (BEQ_{bio} 19 ng/L; BEQ_{chem} 50 ng/L)

Table S2: Studies that have determined the $BEQ_{bio,extract}/BEQ_{chem,nominal}$ ratio to assess chemical SPE recovery expressed as effect in a water matrix.

Study	Extraction Sorbent	Water Matrix	Spiked Chemical Mixture	Bioassay	Endpoint	$BEQ_{bio,extract}/BEQ_{chem,nominal}$
Thorpe *et al.*[7]	C18	Wastewater	Four estrogenic compounds (17β-estradiol, estrone, 17α-ethinylestradiol, nonylphenol)	Recombinant yeast estrogen screen	Activation of ER	1.13 to 1.24
Neale and Escher[8]	Oasis HLB	Treated wastewater	Six herbicides (atrazine, diuron, fluometuron, hexazinone, simazine, terbutryn)	Combined algae assay	2 h photosystem II inhibition	0.91 (BEQ_{bio} 2.03 µg/L; BEQ_{chem} 2.24 µg/L)
Kunz *et al.*[9]	LiChrolut EN-RP18	Ultrapure water	Four estrogenic compounds (17β-estradiol, estrone, 17α-ethinylestradiol bisphenol A)	YES	Activation of ER	1.38 (high mix) (BEQ_{bio} 4.4 ng/L; BEQ_{chem} 3.2 ng/L) 0.76 (low mix) (BEQ_{bio} 0.24 ng/L; BEQ_{chem} 0.32 ng/L)
				ERα CALUX	Activation of ER	0.96 (high mix) (BEQ_{bio} 1.3 ng/L; BEQ_{chem} 1.3 ng/L) 0.98 (low mix) (BEQ_{bio} 0.13 ng/L; BEQ_{chem} 0.13 ng/L)
				T47D-KBluc	Activation of ER	0.32 (high mix) (BEQ_{bio} 1.8 ng/L; BEQ_{chem} 5.6 ng/L) 9.59 (low mix) (BEQ_{bio} 5.4 ng/L; BEQ_{chem} 0.56 ng/L)
				MELN	Activation of ER	0.27 (high mix) (BEQ_{bio} 0.4 ng/L; BEQ_{chem} 1.3 ng/L) 0.34 (low mix) (BEQ_{bio} 0.04 ng/L; BEQ_{chem} 0.13 ng/L)

Study	Extraction Sorbent	Water Matrix	Spiked Chemical Mixture	Bioassay	Endpoint	$BEQ_{bio,extract}/BEQ_{chem,nominal}$
				ERα GeneBLAzer	Activation of ER	0.54 (high mix) (BEQ_{bio} 2.1 ng/L; BEQ_{chem} 3.8 ng/L) 0.64 (low mix) (BEQ_{bio} 0.24 ng/L; BEQ_{chem} 0.38 ng/L)

Section S1: Chemical analysis of LVSPE recovery experiment

For the LC-HRMS screening a Thermo Ultimate 3000 LC system was coupled to a quadrupole-orbitrap instrument (Thermo QExactive Plus) equipped with a heated electrospray ionisation (ESI) source. LC separation was done on a Kinetex C18 EVO column (50 × 2.1 mm, 2.6 µm particle size, Phenomenex) using a gradient elution with 0.1% of formic acid (eluent A) and methanol containing 0.1% of formic acid (eluent B) at a flow rate of 300 µL/min. After 1 min of 5% B, the fraction of B increased linearly to 100% within 12 min and 100% B were kept for 11 min. The eluent flow was diverted to waste and the column was rinsed for 2 min using a mixture of isopropanol + acetone 50:50 / eluent B / eluent A (85% / 10% / 5%) to remove hydrophobic matrix constituents from the column. Finally, the column was re-equilibrated to initial conditions for 5.7 min. The injection volume was 5 µL and the column was operated at 40°C. The heated ESI source and the transfer capillary were both operated at 300 °C, the spray voltage was 3.8 kV (positive mode) or 3.5 kV (negative mode), the sheath gas flow rate was 45 a.u. and the auxiliary gas flow rate 1 a.u. Separate runs were conducted in ESI+ and ESI- mode combining a full scan experiment (80-1000 m/z) at a nominal resolving power of 70,000 (referenced to m/z 200) and data-independent MS/MS experiments at a nominal resolving power of 35,000. For the latter, we acquired the data using broad isolation windows of about 50 Th (i.e., m/z ranges 97-147, 144-194, 191-241, 238-288, 285-335, 332-382, 379-429, 426-476) and 280 Th (i.e., m/z ranges 460-740, 730-1010), respectively.
Compounds were quantified against reference standards based on extracted ion chromatograms with a 7 ppm window around the theoretical mass using matrix-matched, internal calibration with 40 isotope-labelled internal standards. These were spiked prior to analysis and the internal standard with the closest retention time was chosen for quantification of a target compound. For small peaks or those showing a high background noise, compound identity was verified using one to three diagnostic MS/MS fragment ions per compound. Data evaluation was done with the TraceFinder 3.3 software (Thermo).

For target analysis of phenolic and other compounds (see Table S4) with poor ionization in the HRMS screening method an Agilent 1260 LC system coupled to an ABSciex QTrap 6500 MS was used. Gradient elution was done on Kinetex C18 column (100 x 3.0 mm, 2.6 µm particle size, Phenomenex) using 1 mM ammonium fluoride (A) and methanol (B) at a flow rate of 0.35 mL/min at 30°C (based on the method by Griffith *et al.*[10]). The gradient started at 20% B and was held for 1 min, before linearly increasing to 90% B for 4 min, and to 95% B for 9 min, where it was held for 2.2 min. Re-equilibration was done for 5 min. The injection volume was 10 µL. For ionization a

Turbo V ion source was operated in ESI- mode with the following settings: spray voltage -3.6 kV, temperature 380°C, nebulizer gas 60 psig, heater gas 60 psig, curtain gas 50 psig, and entrance potential -10.0 V. Two MS/MS transitions per compound were recorded in scheduled multiple reaction monitoring (sMRM) mode. For quantification, internal, matrix-matched calibration was used with isotope-labelled compounds. For data evaluation the software Multiquant 3.0 (ABSciex) was used.

Section S2: Chemical analysis of multi-layer SPE recovery experiments

Analysis of the multi-layer SPE cartridge extracts was performed on a high performance liquid chromatography (HPLC) coupled to a QExactive HRMS (Thermo). The HPLC consisted of a CTC Pal auto sampler (CTC analytics, Zwingen/Switzerland), a RHEOS 2200 pump with degasser (Flux Instruments, Switzerland) and a column oven. The separation of the analytes was realized with a reversed phase column (XBridge™ C18 column; 3.5 µm, 2.1 x 50 mm; Waters, U.S.) coupled with a pre-column (3.5 µm, 2.1 x 10 mm). The mobile phase consisted of water and methanol both acidified with formic acid (0.1%). The initial ratio was set to 90:10 water:methanol and throughout a runtime of 29 minutes different ratios up to 5:95 were realized. The flow rate was set to 200 µL/min and the injection volume to 30 µL.

The background sample was determined three times and recovery samples were prepared three times with a 200, a 400 and a 600 ng absolute spike; so a linear regression was based on four distributed points. Compound recovery was calculated as the difference in concentration of the spike after SPE sample and the spike before SPE sample. The three obtained ratios were plotted against the theoretic concentration. Absolute recovery was determined by dividing the slope of the background-spike after SPE curve over the background-spike before SPE curve.

Figure S1: Ratio of $f_{recovery,i}$ for LVSPE from the current study and $f_{recovery,i}$ for LVSPE (HR-X only) from Schulze *et al.*[1] and ratio of $f_{recovery,i}$ for LVSPE and $f_{recovery,i}$ for multi-layer SPE (both measured in current study).

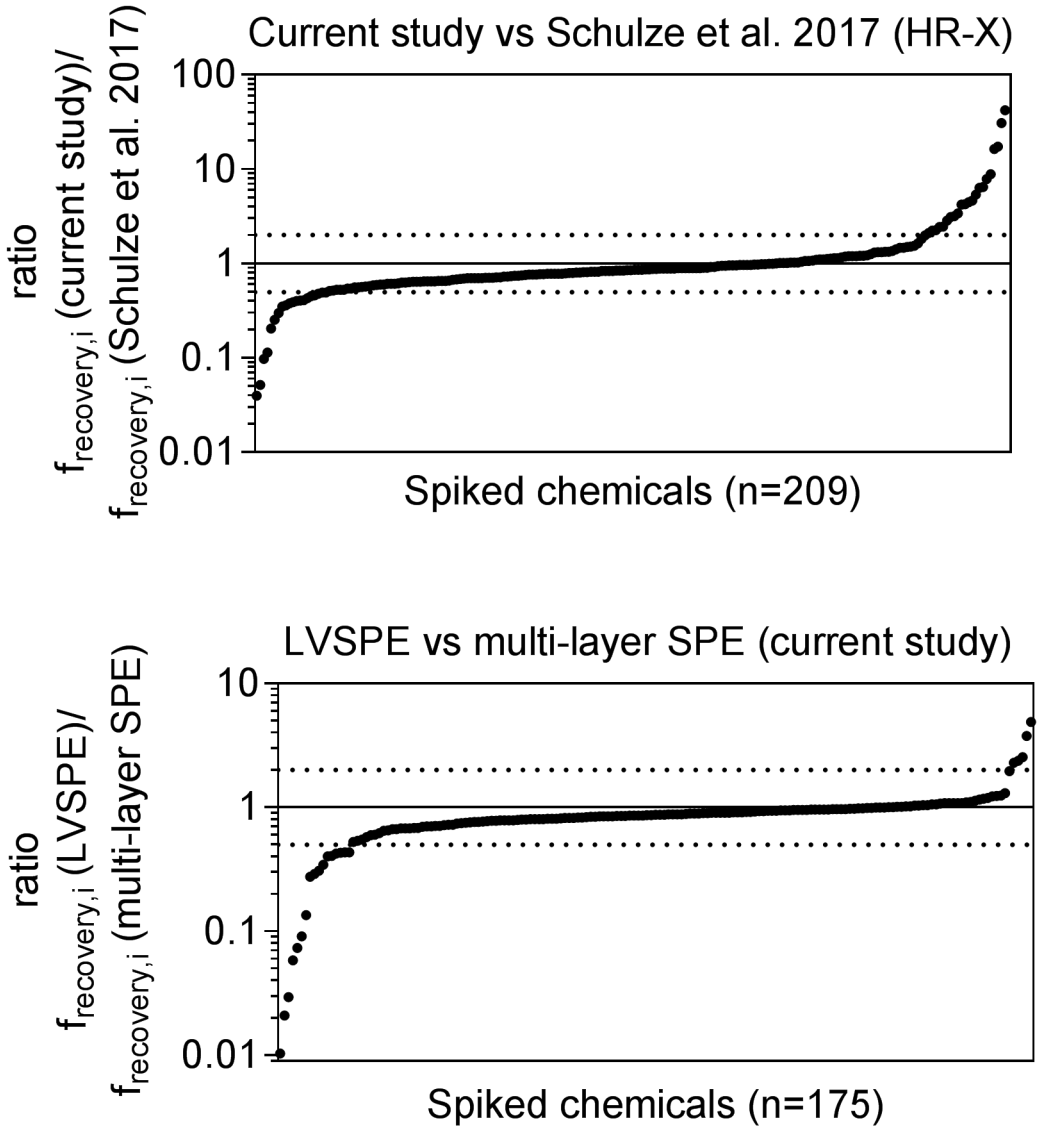


Table S6: EC values (REF) ± standard error for unspiked Wormsgraben water (water), mix stock solution (mix) and spiked Wormsgraben water (water+mix).

Assay	Water extract	Mix	Water + mix extract	LVSPE blank	Solvent blank
AhR CALUX (EC_{10})	23.8 ± 0.08	3.64 ± 0.15	4.72 ± 0.24	>250	>1000
HG5LN-hPXR (EC_{10})	36.2 ± 5.15	54.5 ± 9.11	3.75 ± 0.76	>50	>50
PPARγ GeneBLAzer (EC_{10})	33.5 ± 3.04	5.40 ± 0.25	3.19 ± 0.30	>80	>80
MELN (EC_{10})	36.2 ± 5.15	0.10 ± 0.01	0.08 ± 0.01	>50	>50
ER GeneBLAzer (EC_{10})	>30	0.59 ± 0.01	1.68 ± 0.06	>150	>150
AR GeneBLAzer (EC_{10})	>90	6.83 ± 0.12	5.47 ± 0.12	>150	>150
GR GeneBLAzer (EC_{10})	>30	4.82 ± 0.10	6.83 ± 0.28	>150	>150
PR GeneBLAzer (EC_{10})	>30	3.40 ± 0.06	5.14 ± 0.17	>150	>150
AREc32 ($EC_{IR1.5}$)	22.9 ± 0.58	129 ± 3.64	16.2 ± 0.40	>65	>65
FET (EC_{50}) (95% CI)	100 (91.9 to 113)	84.6 (80.7 to 88.7)	103 (91.9 to 121)	>120	-

Figure S2: Full concentration-effect curves for induction (blue filled symbols) and cell viability (empty symbols) for AhR CALUX (left plots), with linear concentration-effect curves for induction (right plots).

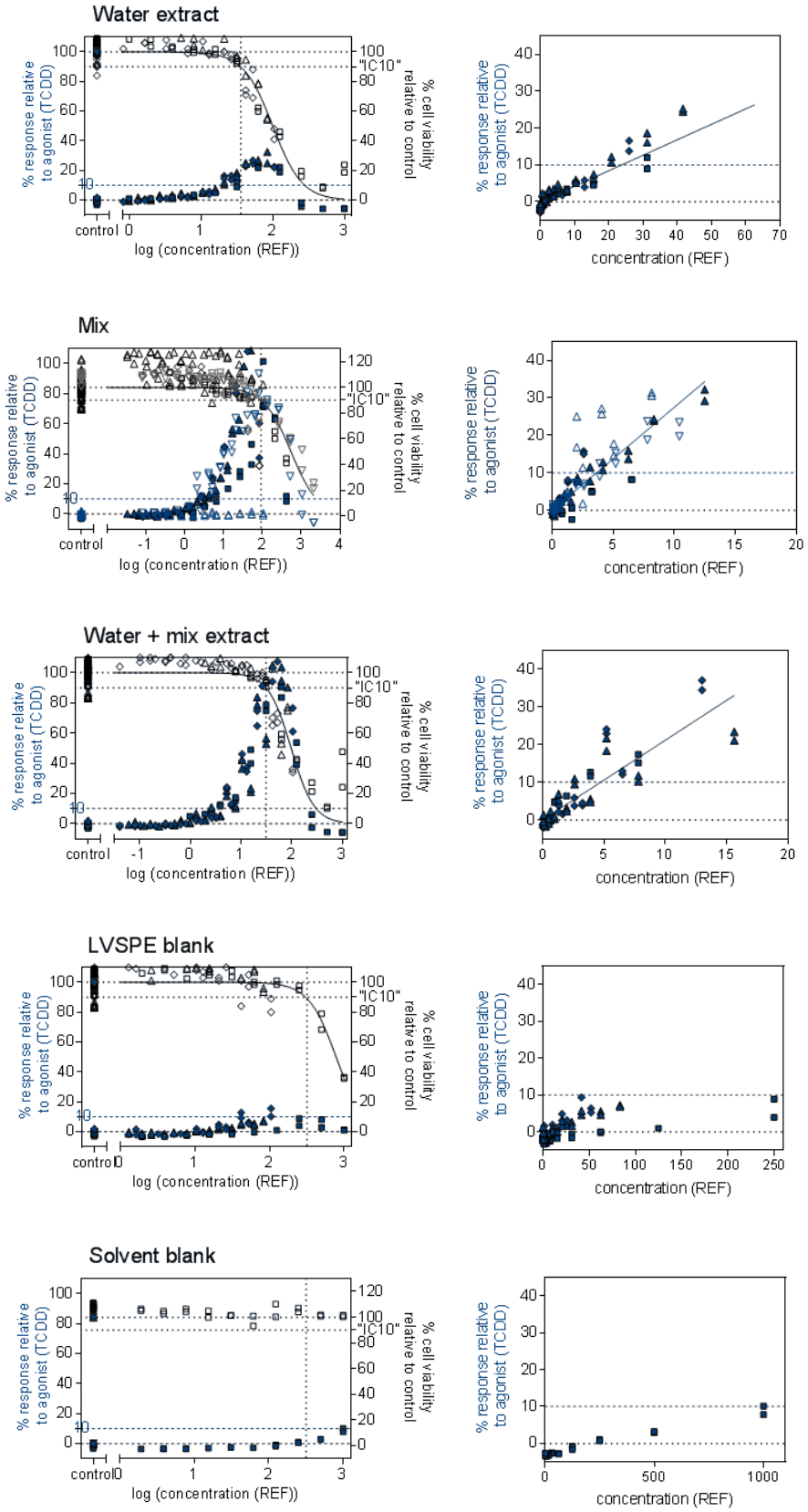


Figure S3: Full concentration-effect curves for induction for HG5LN-hPXR (left plots), with linear concentration-effect curves for induction (right plots).

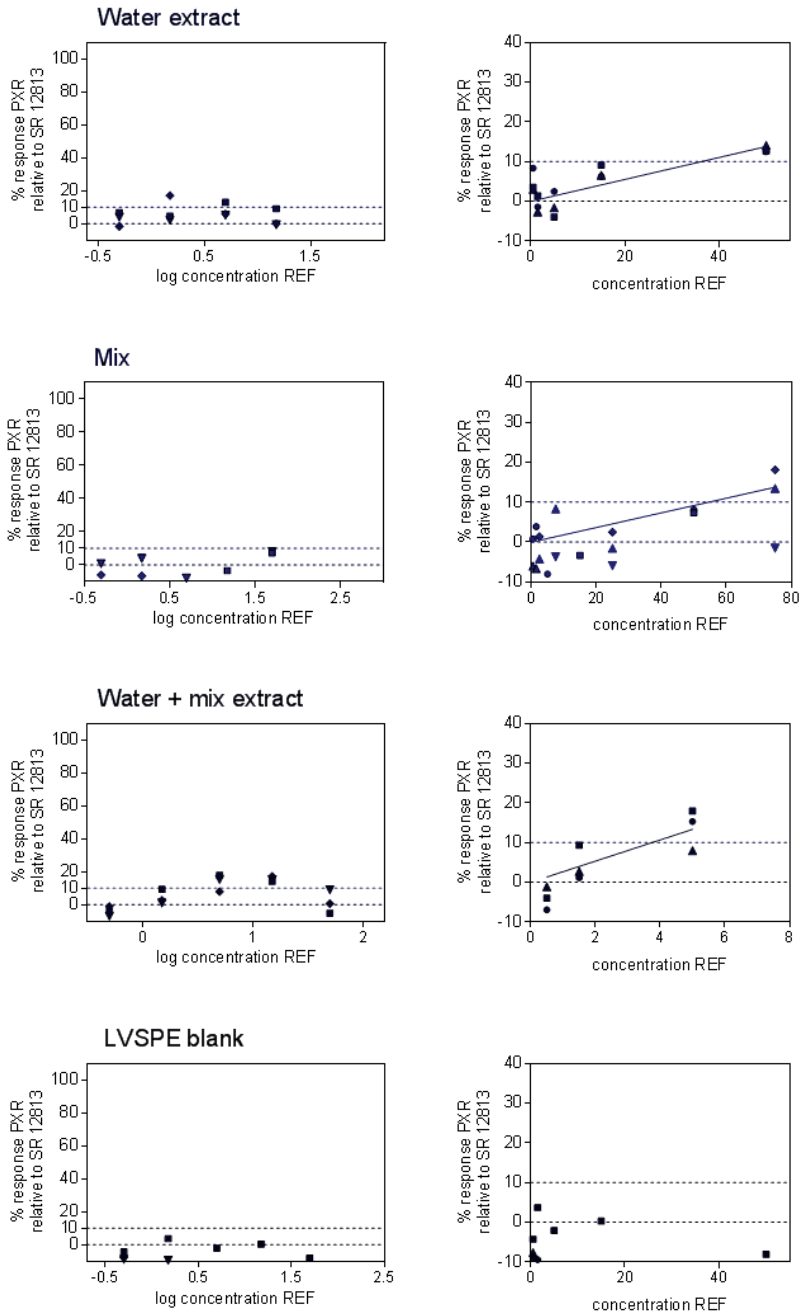


Figure S4: Full concentration-effect curves for induction (blue filled symbols) and cell viability (empty symbols) for PPARγ GeneBLAzer (left plots), with linear concentration-effect curves for induction (right plots).

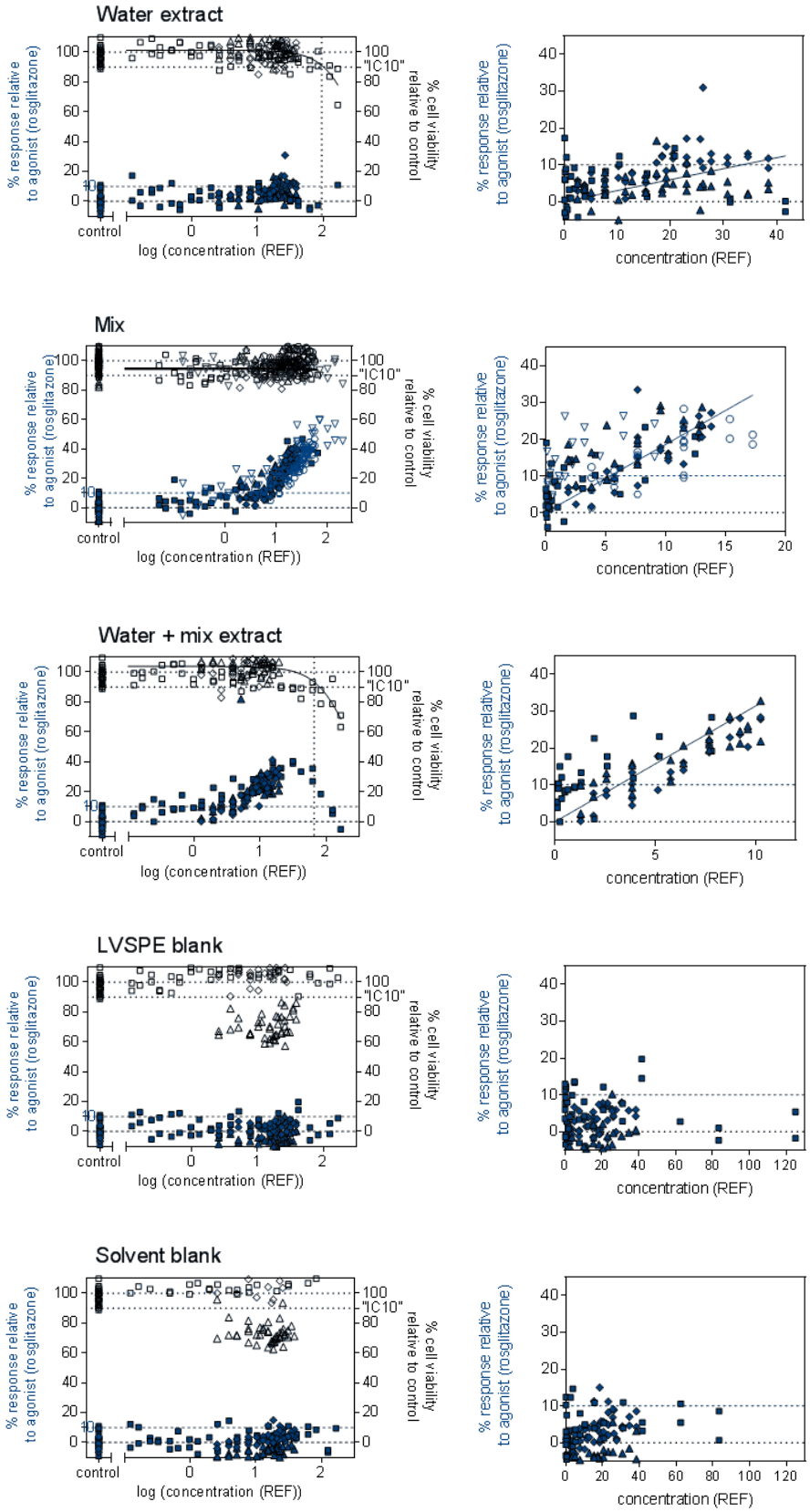


Figure S5: Full concentration-effect curves for induction for MELN (left plots), with linear concentration-effect curves for induction (right plots).

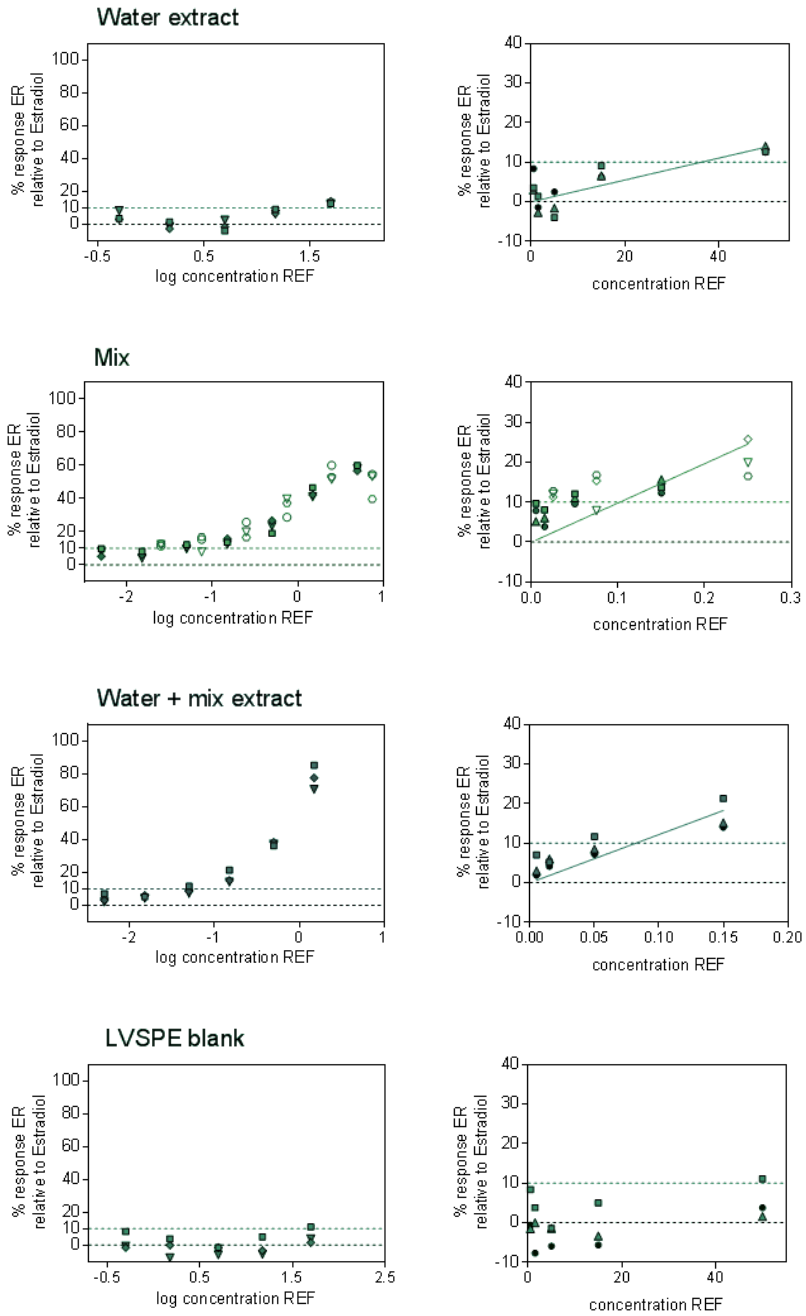


Figure S6: Full concentration-effect curves for induction (green filled symbols) and cell viability (empty symbols) for ER GeneBLAzer (left plots), with linear concentration-effect curves for induction (right plots).

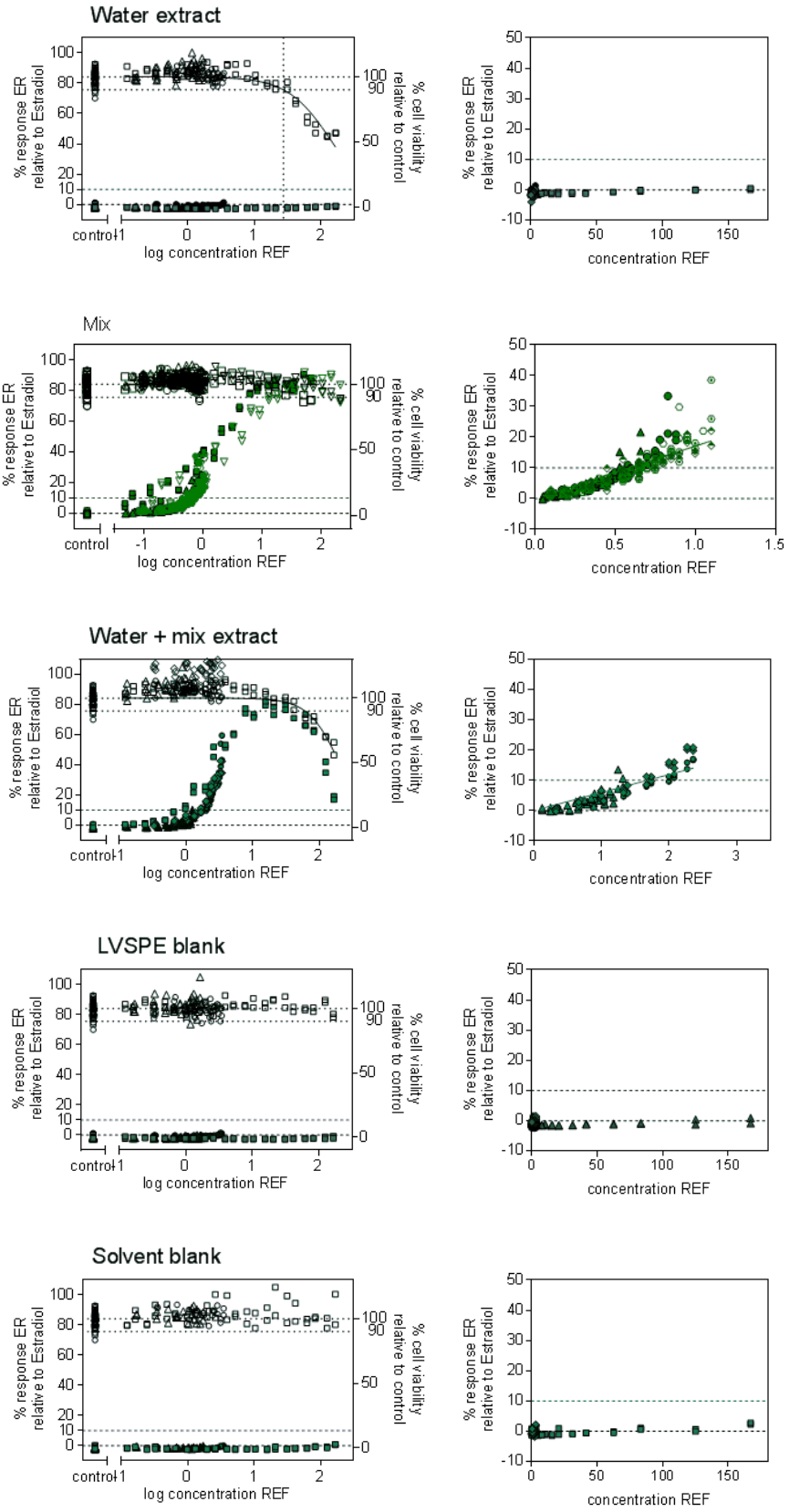


Figure S7: Full concentration-effect curves for induction (green filled symbols) and cell viability (empty symbols) for AR GeneBLAzer (left plots), with linear concentration-effect curves for induction (right plots).

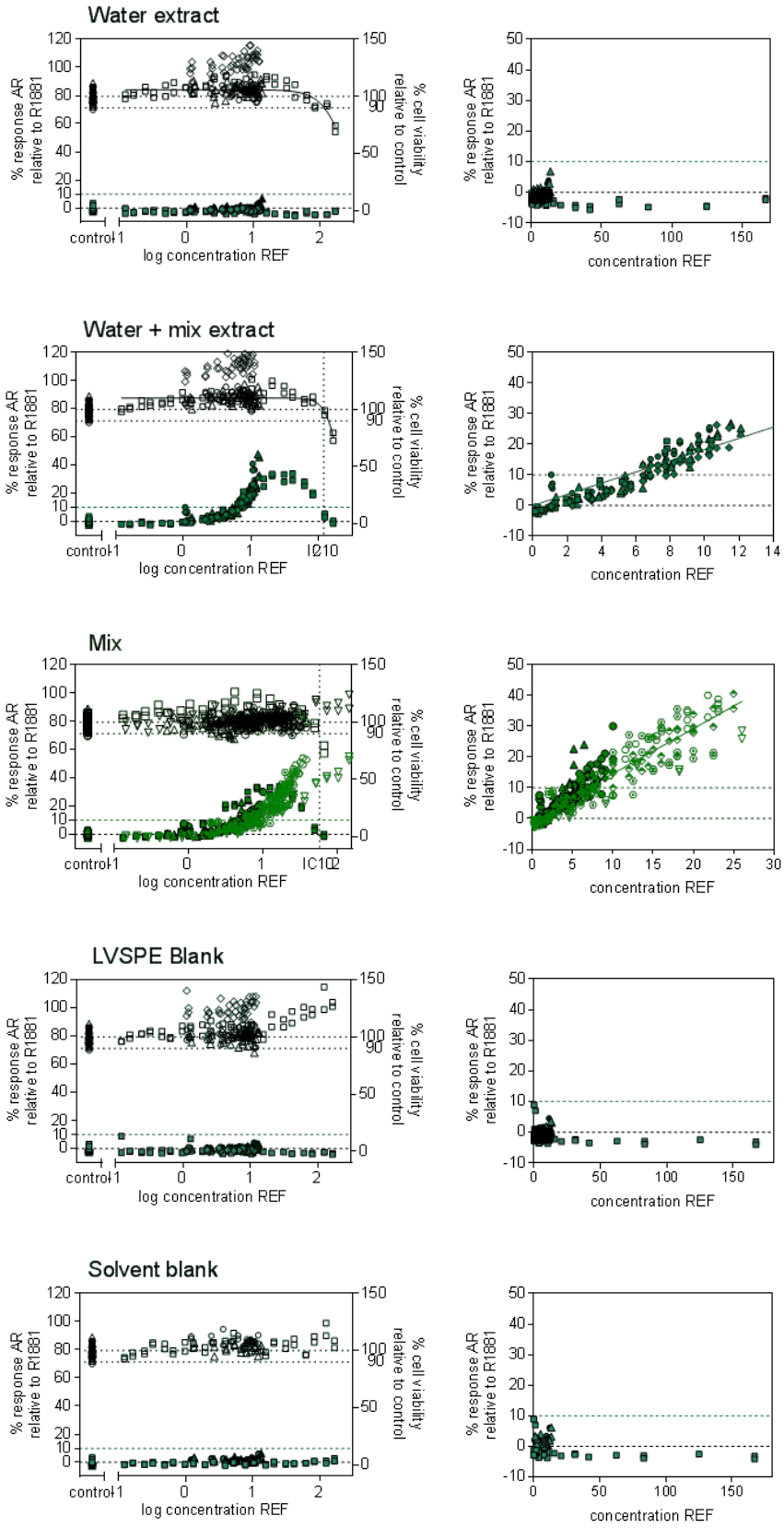


Figure S8: Full concentration-effect curves for induction (green filled symbols) and cell viability (empty symbols) for GR GeneBLAzer (left plots), with linear concentration-effect curves for induction (right plots).

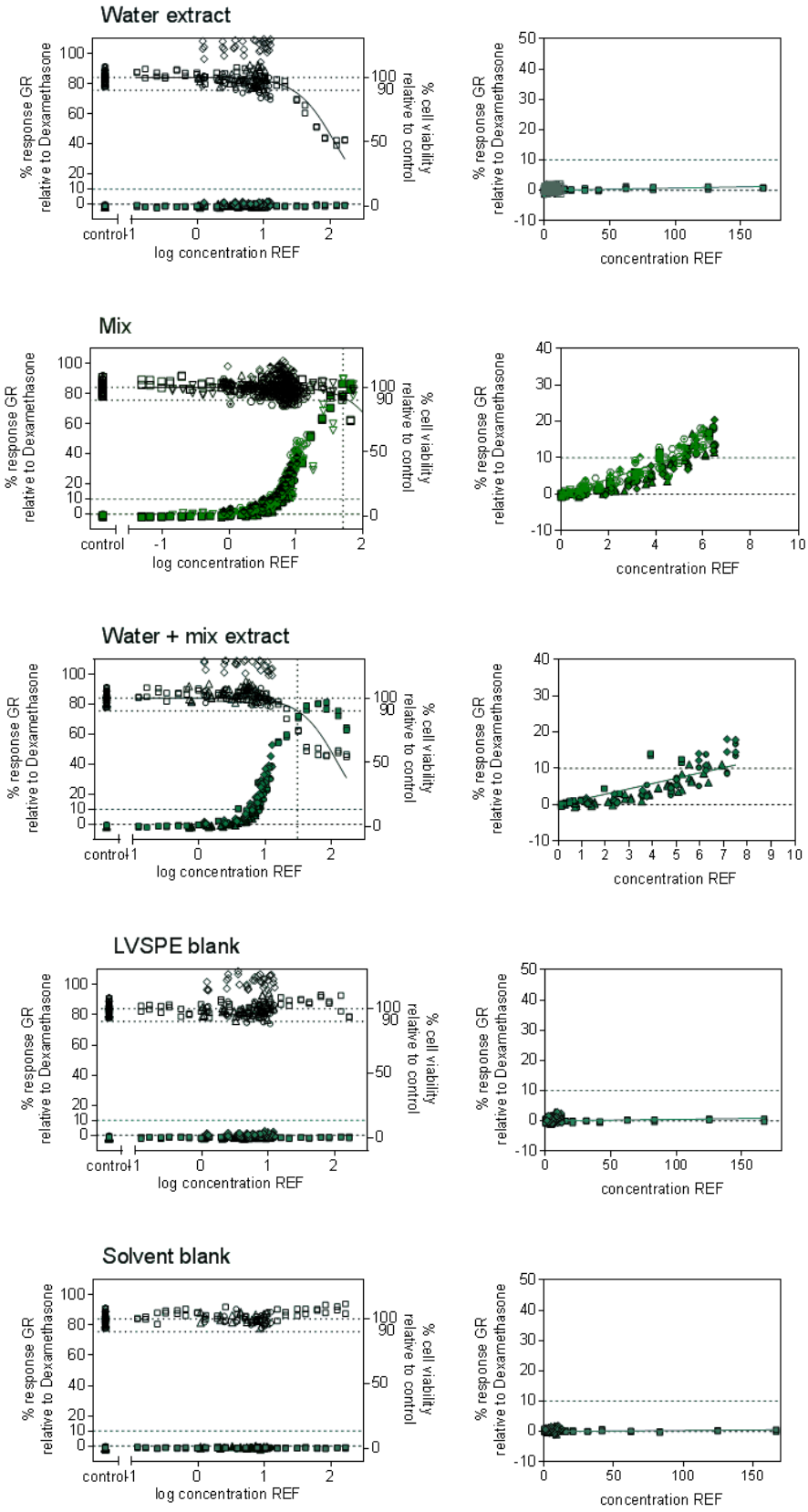


Figure S9: Full concentration-effect curves for induction (green filled symbols) and cell viability (empty symbols) for PR GeneBLAzer (left plots), with linear concentration-effect curves for induction (right plots).

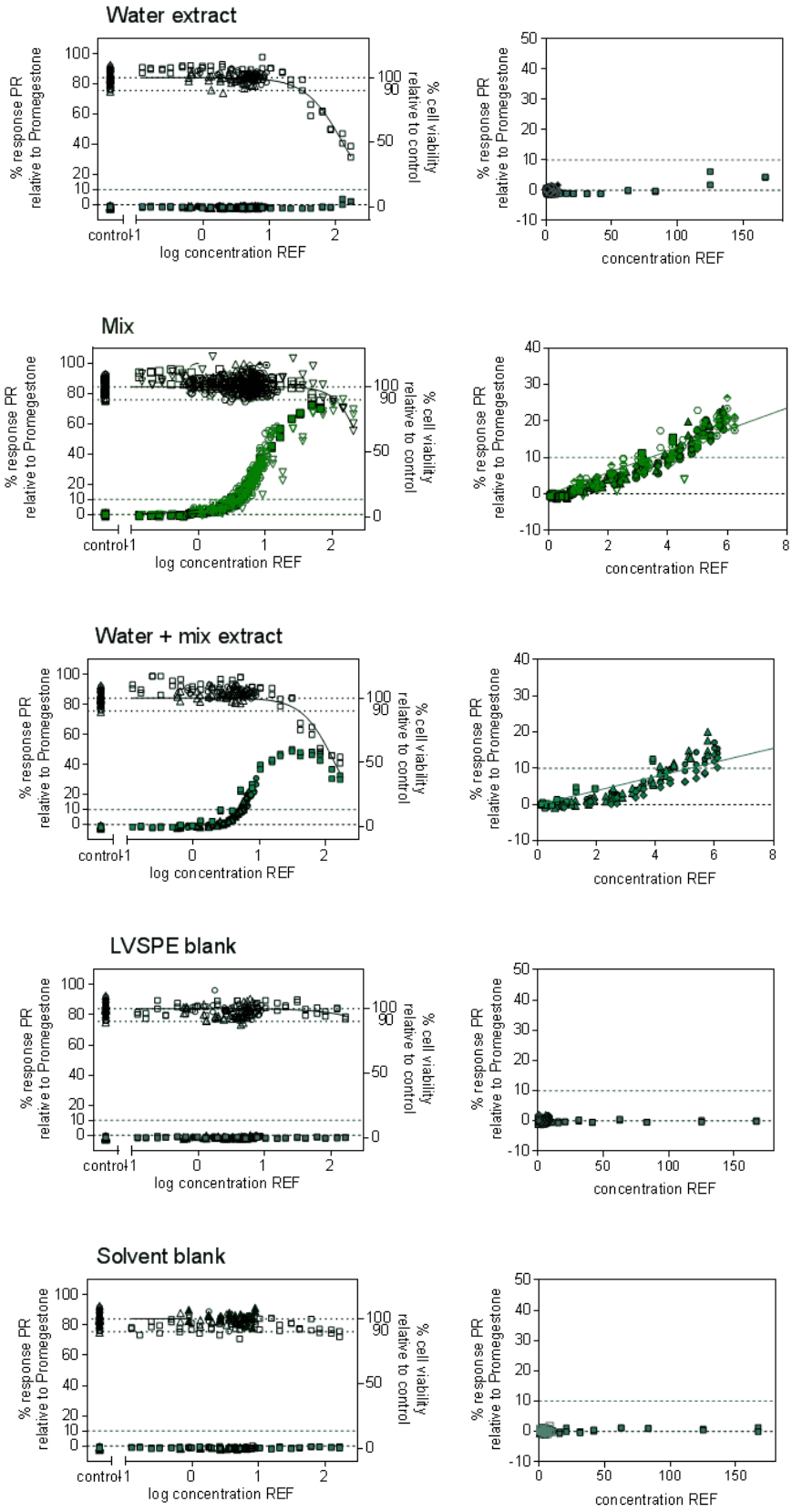


Figure S10: Full concentration-effect curves for induction (red filled symbols) and cell viability (empty symbols) for AREc32 (left plots), with linear concentration-effect curves for induction (right plots).

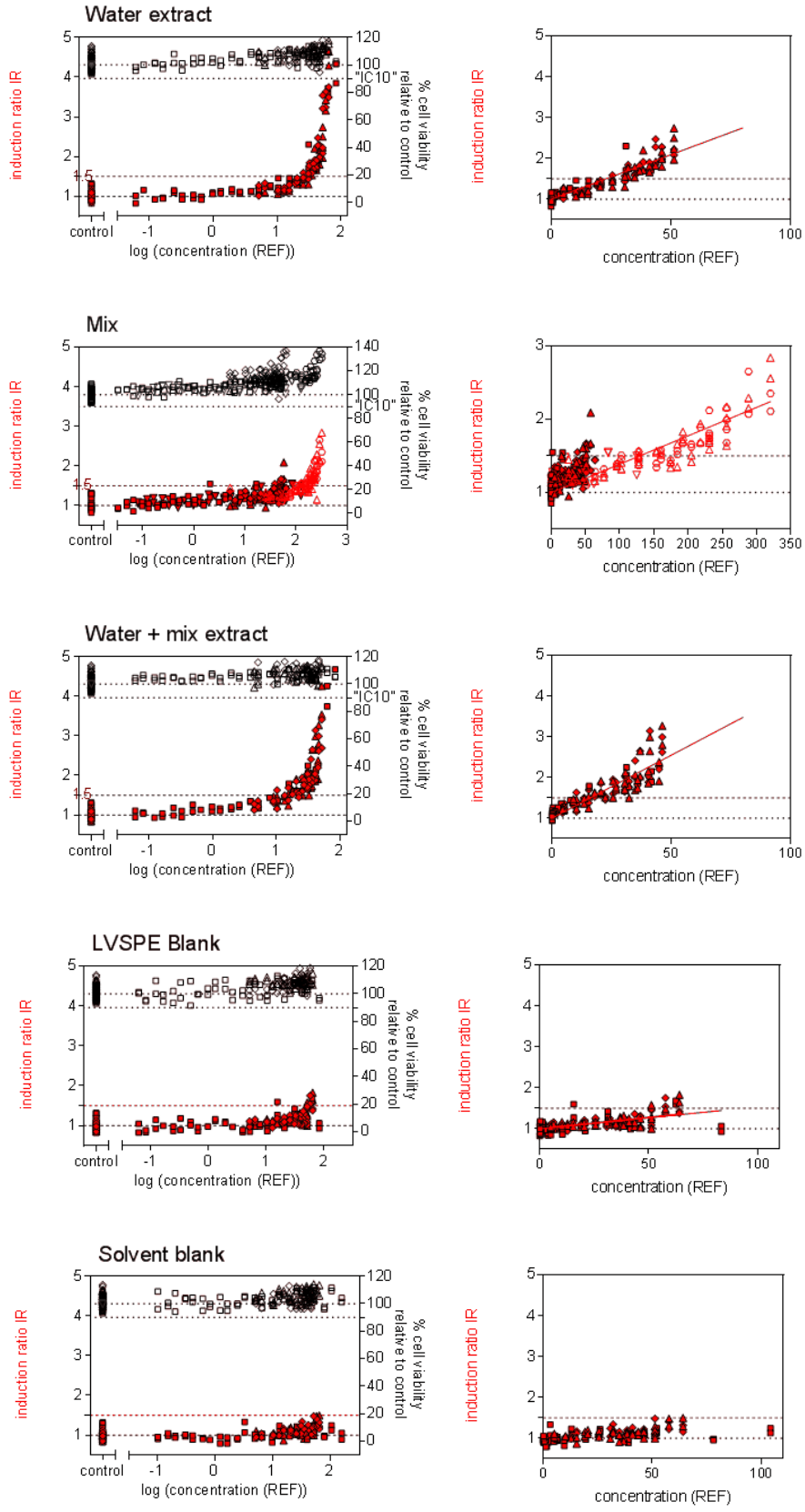


Figure S11: Full concentration-effect curves for fish embryo toxicity test. Up to 33% mortality was observed in the LVSPE blank at REF 60. This is expected to be an artefact, potentially due to contaminated glassware, as all three embryos in the same vial were dead, but embryos in the other vials at REF 60 were alive.

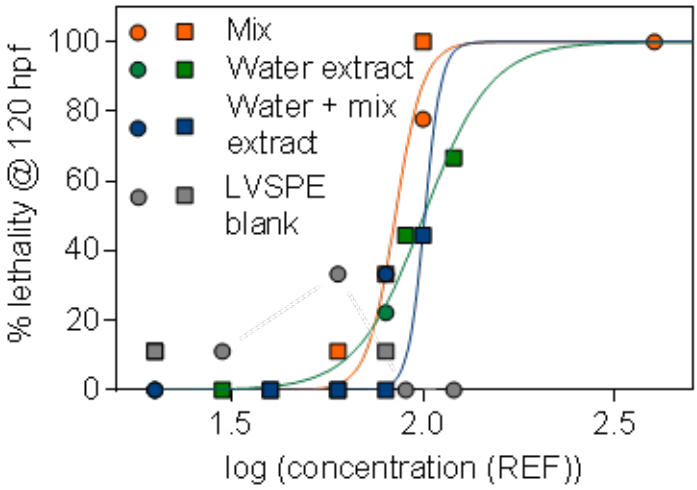


Table S7: EC values (REF) for the process blanks in the multilayer SPE. The original multilayer SPE (A+B), without Envicarb (C+D), with only Oasis HLB (E+F) conditioned with methanol (MeOH) (A,C,E) and methanol (MeOH):ethyl acetate (EtAc) (1:1) (B, D, F). Standard error provided for all assays, except for fish embryo toxicity, where 95% confidence intervals are provided.

AhR CALUX			
SPE material	**solvent**	**IC_{10} (REF) (cytotoxicity)**	**EC_{10} (REF)**
A. OasisHLB+StratEnv+Carb	MeOH	275	161 ± 17
B. OasisHLB+StratEnv+Carb	EtAc/MeOH	21	>21
C. OasisHLB+StratEnv	MeOH	260	145 ± 21
D. OasisHLB+StratEnv	EtAc/MeOH	254	>254
E. Oasis HLB	MeOH	>35	16.4 ± 2.7
F. Oasis HLB	EtAc/MeOH	17	>17

PPARγ GeneBLAzer			
SPE material	**solvent**	**IC_{10} (REF) (cytotoxicity)**	**EC_{10} (REF)**
A. OasisHLB+StratEnv+Carb	MeOH	25	10.4 ± 2.1
B. OasisHLB+StratEnv+Carb	EtAc/MeOH	23	16.7 ± 3.3
C. OasisHLB+StratEnv	MeOH	16	>10
D. OasisHLB+StratEnv	EtAc/MeOH	22	>20
E. Oasis HLB	MeOH	18	>10
F. Oasis HLB	EA/MeOH	46	15.4±1.6

ER GeneBLAzer			
SPE material	**solvent**	**IC_{10} (REF) (cytotoxicity)**	**EC_{10} (REF)**
A. OasisHLB+StratEnv+Carb	MeOH	27	>27
B. OasisHLB+StratEnv+Carb	EtAc/MeOH	38	>38
C. OasisHLB+StratEnv	MeOH	52	>110
D. OasisHLB+StratEnv	EtAc/MeOH	111	>111
E. Oasis HLB	MeOH	50	>50
F. Oasis HLB	EtAc/MeOH	94	>94

AR GeneBLAzer			
SPE material	**solvent**	**IC_{10} (REF) (cytotoxicity)**	**EC_{10} (REF)**
A. OasisHLB+StratEnv+Carb	MeOH	43	>43
B. OasisHLB+StratEnv+Carb	EtAc/MeOH	18	>18
C. OasisHLB+StratEnv	MeOH	23	>23
D. OasisHLB+StratEnv	EtAc/MeOH	30	>30
E. Oasis HLB	MeOH	34	>34
F. Oasis HLB	EtAc/MeOH	23	>23

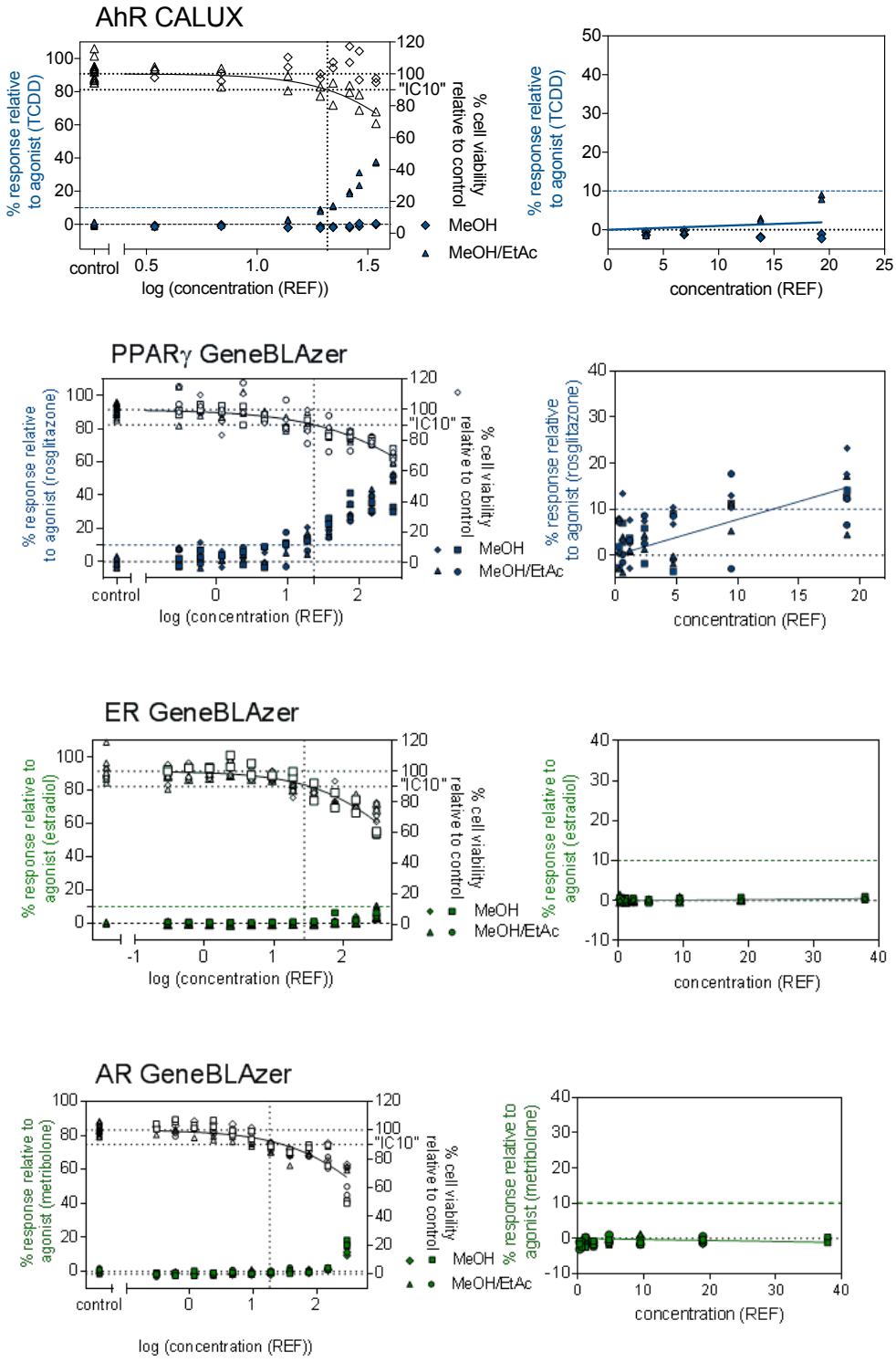
GR GeneBLAzer			
SPE material	**solvent**	**IC_{10} (REF) (cytotoxicity)**	**EC_{10} (REF)**
A. OasisHLB+StratEnv+Carb	MeOH	41	>41
B. OasisHLB+StratEnv+Carb	EtAc/MeOH	57	>57
C. OasisHLB+StratEnv	MeOH	34	>34
D. OasisHLB+StratEnv	EtAc/MeOH	36	>36
E. Oasis HLB	MeOH	36	>36
F. Oasis HLB	EtAc/MeOH	35	>35

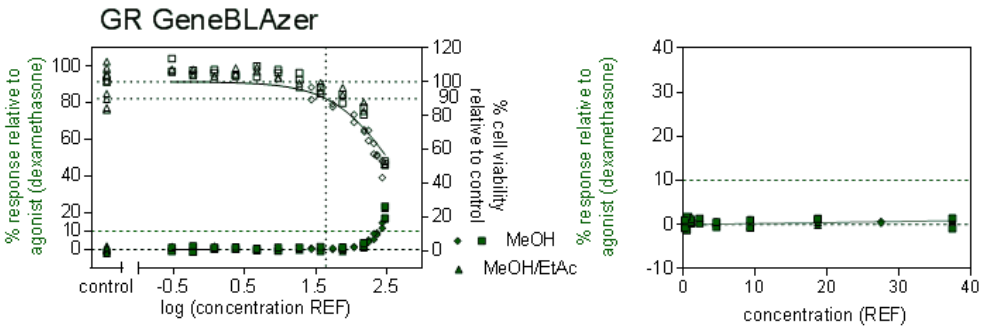
PR GeneBLAzer			
SPE material	**solvent**	**IC_{10} (REF) (cytotoxicity)**	**EC_{10} (REF)**
A. OasisHLB+StratEnv+Carb	MeOH	73	>73
B. OasisHLB+StratEnv+Carb	EtAc/MeOH	126	>126
C. OasisHLB+StratEnv	MeOH	68	>68
D. OasisHLB+StratEnv	EtAc/MeOH	105	>105
E. Oasis HLB	MeOH	59	>59
F. Oasis HLB	EtAc/MeOH	61	>61

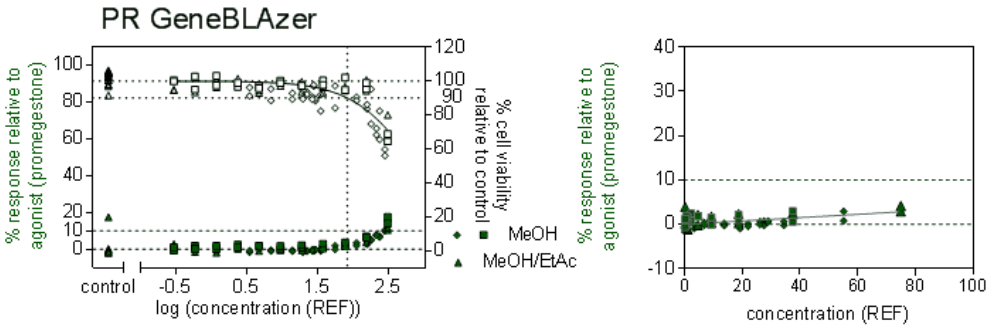
AREc32			
SPE material	**solvent**	**IC_{10} (REF) (cytotoxicity)**	**$EC_{IR1.5}$ (REF)**
A. OasisHLB+StratEnv+Carb	MeOH	>300	36.6 ± 1.2
B. OasisHLB+StratEnv+Carb	EtAc/MeOH	>300	39.6 ± 2.0
C. OasisHLB+StratEnv	MeOH	>300	31.8 ± 1.2
D. OasisHLB+StratEnv	EtAc/MeOH	>300	37.4 ± 1.8
E. Oasis HLB	MeOH	233	25.8 ± 0.8
F. Oasis HLB	EtAc/MeOH	>300	44.7 ± 2.0

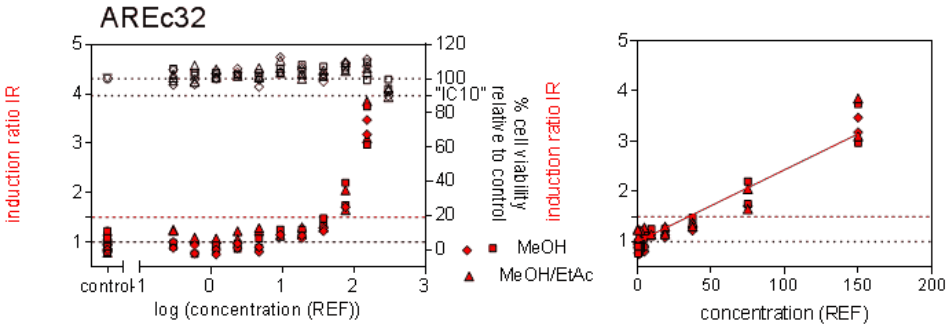
Fish embryo toxicity				
SPE material	**solvent**	**EC_{50} 24 h (REF)**	**EC_{50} 48 h (REF)**	**EC_{50} 120 h (REF)**
A. OasisHLB+StratEnv+Carb	MeOH	>67	>67	33.3 (21.5 - 64.0)
B. OasisHLB+StratEnv+Carb	EtAc/MeOH	>67	>67	37.6 (31.6 - 46.7)
C. OasisHLB+StratEnv	MeOH	>67	>67	47.3 (33.8 – 140)
D. OasisHLB+StratEnv	EtAc/MeOH	>67	>67	58.9 (53.6 – 62.8)
E. Oasis HLB	MeOH	>67	>67	29.2 (17.8 – 45.3)
F. Oasis HLB	EtAc/MeOH	>67	>67	40.4 (35.7 – 49.4)

Figure S12: Full concentration-effect curves of the process blanks in the multilayer SPE conditioned with methanol (MeOH) and methanol and ethyl acetate (MeOH/EtAc) for induction (filled symbols) and cell viability (empty symbols) (left plots), with linear concentration-effect curves for induction (right plots).




GR GeneBLAzer
% response relative to agonist (dexamethasone)
% cell viability relative to control
MeOH
MeOH/EtAc
control
log (concentration REF)
concentration (REF)


PR GeneBLAzer
% response relative to agonist (promegestone)
% cell viability relative to control
MeOH
MeOH/EtAc
control
log (concentration (REF))
concentration (REF)


AREc32
induction ratio IR
% cell viability relative to control
"IC10"
MeOH
MeOH/EtAc
control
log (concentration (REF))
concentration (REF)

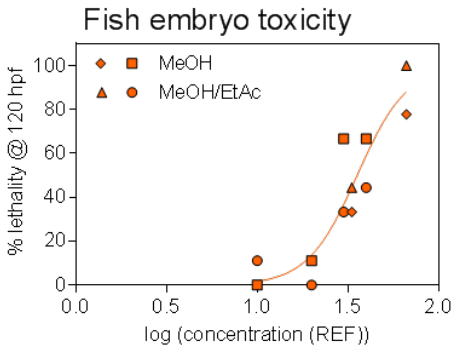

Fish embryo toxicity
% lethality @ 120 hpf
MeOH
MeOH/EtAc
log (concentration (REF))

Table S8: EC values and REP_i values for AhR CALUX, HG5LN-hPXR, PPARγ GeneBLAzer and AREc32.

	AhR CALUX		**HG5LN-hPXR**		**PPARγ GeneBLAzer**		**AREc32**	
	EC_{10} (M)	REP_i	EC_{10} (M)	REP_i	EC_{10} (M)	REP_i	$EC_{IR1.5}$ (M)	REP_i
1,2-Benzisothiazolinone					1.27×10^{-5d}	7.77×10^{-5}		
17α-Ethinylestradiol			3.34×10^{-7b}	4.22×10^{-2}				
2-Mercaptobenzothiazole					1.19×10^{-5d}	8.29×10^{-5}		
3-Iodopropynyl butylcarbamate					9.75×10^{-5d}	1.01×10^{-5}		
4-n-Nonylphenol	1.36×10^{-5a}	4.17×10^{-8}					6.73×10^{-5a}	2.86×10^{-2}
7-Diethylamino-4-methylcoumarin					1.51×10^{-6d}	6.54×10^{-4}		
Abamectin					4.59×10^{-6d}	2.15×10^{-4}		
Acetaminophen							3.63×10^{-3e}	5.31×10^{-4}
Amitraz							1.02×10^{-4e}	1.89×10^{-2}
Atenolol							7.24×10^{-4e}	2.66×10^{-3}
Atorvastatin							3.11×10^{-5e}	6.20×10^{-2}
Atrazine							1.05×10^{-4e}	1.84×10^{-2}
Bezafibrate			2.24×10^{-5b}	6.30×10^{-4}	6.22×10^{-6d}	1.59×10^{-4}		
Bisphenol A			4.75×10^{-6a}	2.97×10^{-3}			1.24×10^{-4a}	1.55×10^{-2}
Carbamazepine			3.63×10^{-5a}	3.89×10^{-4}				
Chlorophene			1.02×10^{-5a}	1.38×10^{-3}			7.49×10^{-5a}	2.57×10^{-2}
Citalopram							2.95×10^{-4e}	6.54×10^{-3}
Clotrimazole			5.23×10^{-8b}	2.70×10^{-1}	5.99×10^{-6d}	1.65×10^{-4}		
Cyprodinil	5.00×10^{-6a}	1.14×10^{-7}	5.26×10^{-6a}	2.68×10^{-3}			7.11×10^{-4a}	2.71×10^{-3}
Diazepam			9.06×10^{-6b}	1.56×10^{-3}				
Diazinon	1.35×10^{-5a}	4.21×10^{-8}	1.44×10^{-6a}	9.80×10^{-3}	5.30×10^{-5a}	1.86×10^{-5}		
Dichlorvos							7.70×10^{-6e}	2.50×10^{-1}

	AhR CALUX		HG5LN-hPXR		PPARγ GeneBLAzer		AREc32	
	EC_{10} (M)	REP_i	EC_{10} (M)	REP_i	EC_{10} (M)	REP_i	$EC_{IR1.5}$ (M)	REP_i
Diclofenac			3.78×10^{-5a}	3.73×10^{-4}	2.55×10^{-6a}	3.87×10^{-4}		
Diuron			1.14×10^{-4a}	1.24×10^{-4}				
Erythromycin			4.78×10^{-4c}	2.95×10^{-5}				
Estrone			3.56×10^{-6c}	3.96×10^{-3}				
Fenofibrate			1.34×10^{-7b}	1.05×10^{-1}				
Fipronil			6.35×10^{-6a}	2.22×10^{-3}			2.12×10^{-5e}	9.09×10^{-2}
Flufenoxuron					7.49×10^{-6d}	1.32×10^{-4}		
Flutamide			2.17×10^{-6a}	6.50×10^{-3}				
Genistein			1.24×10^{-6a}	1.14×10^{-2}			7.15×10^{-5a}	2.70×10^{-2}
Hexadecylpyridinium			1.00×10^{-6a}	1.41×10^{-2}				
Indometacin					6.43×10^{-7d}	1.53×10^{-3}		
Isoproturon			8.33×10^{-6c}	1.69×10^{-3}				
Ketoconazole					1.81×10^{-5d}	5.45×10^{-5}		
Ketoprofen			3.88×10^{-5b}	3.64×10^{-4}				
Linuron			4.09×10^{-6b}	3.45×10^{-3}				
Losartan					4.77×10^{-6d}	2.07×10^{-4}		
Mefenamic acid			1.08×10^{-5a}	1.31×10^{-3}				
Metolachlor			2.68×10^{-7a}	5.26×10^{-2}				
Metoprolol							3.66×10^{-4e}	5.27×10^{-3}
Naproxen							3.70×10^{-3e}	5.21×10^{-4}
Oxadiazone			5.90×10^{-8b}	2.39×10^{-1}				
Picoxystrobin			1.73×10^{-5a}	8.15×10^{-4}				
Propiconazole			2.94×10^{-6a}	4.80×10^{-3}			4.05×10^{-5e}	4.76×10^{-2}
Propranolol							2.59×10^{-5e}	7.44×10^{-2}

	AhR CALUX		HG5LN-hPXR		PPARγ GeneBLAzer		AREc32	
	EC_{10} (M)	REP_i	EC_{10} (M)	REP_i	EC_{10} (M)	REP_i	$EC_{IR1.5}$ (M)	REP_i
Quinoxyfen					1.45×10^{-5}[d]	6.81×10^{-5}		
Raloxifene					2.72×10^{-5}[d]	3.63×10^{-5}		
Ranitidine							2.04×10^{-3}[e]	9.45×10^{-4}
Tamoxifen			1.40×10^{-7}[b]	1.01×10^{-1}	5.05×10^{-6}[d]	1.95×10^{-4}		
Tebuconazole			1.88×10^{-6}[a]	7.50×10^{-3}				
Telmisartan	1.57×10^{-5}[a]	3.62×10^{-8}			1.43×10^{-7}[a]	6.90×10^{-3}		
Terbuthylazine			1.03×10^{-5}[a]	1.37×10^{-3}				
Tetrachlorosalicylanilide					2.68×10^{-5}[d]	3.68×10^{-5}		
Triclocarban							4.81×10^{-6}[a]	4.01×10^{-1}
Triclosan			1.77×10^{-6}[a]	7.97×10^{-3}			1.71×10^{-5}[a]	1.13×10^{-1}
Triphenylphosphate			9.10×10^{-7}[a]	1.55×10^{-2}	4.24×10^{-6}[a]	2.33×10^{-4}		

[a]Neale *et al.*[11]; [b]Creusot *et al.*[12]; [c]Creusot[13]; [d]US EPA[14]; [e]Escher *et al.*[15]

Table S9: EC values and REP_i values for MELN, ER GeneBLAzer, AR GeneBLAzer and GR GeneBLAzer.

	MELN		**ER GeneBLAzer**		**AR GeneBLAzer**		**GR GeneBLAzer**	
	EC_{10} (M)	REP_i	EC_{10} (M)[j]	REP_i	EC_{10} (M)[j]	REP_i	EC_{10} (M)[j]	REP_i
1,2-Benzisothiazolinone							8.96×10^{-6}	9.48×10^{-5}
17α-Estradiol		2.00×10^{-2}[a]	2.45×10^{-10}	1.02×10^{-1}	1.76×10^{-7}	1.09×10^{-3}		
17α-Ethinylestradiol	7.93×10^{-13}[b]	3.05×10^{0}	9.21×10^{-11}	2.71×10^{-1}				
17α-Hydroxyprogesterone			2.15×10^{-6}	1.16×10^{-5}	3.58×10^{-10}	5.34×10^{-1}		
17α-Methyltestosterone			1.36×10^{-6}	1.84×10^{-5}	3.65×10^{-10}	5.23×10^{-1}		
17β-Estradiol	2.42×10^{-12}[c]	1.00×10^{0}	2.50×10^{-11}[c]	1.00×10^{0}	1.41×10^{-7}	1.36×10^{-3}		
2,2-Dimethoxy-2-phenylacetophenone			3.87×10^{-6}	6.46×10^{-6}				
4-Androstene-3,17-dione		9.7×10^{-7}[d]			1.21×10^{-8}	1.58×10^{-2}		
4-Cumylphenol			1.80×10^{-6}	1.39×10^{-5}			6.01×10^{-6}	1.41×10^{-4}
4-Hydroxytamoxifen							2.05×10^{-6}	4.14×10^{-4}
4-n-Nonylphenol	9.69×10^{-7}[e]	2.49×10^{-6}						
4-n-Octylphenol	6.02×10^{-9}[b]	4.01×10^{-4}						
7-Diethylamino-4-methylcoumarin			2.80×10^{-6}	8.91×10^{-6}	7.45×10^{-7}	2.56×10^{-4}	5.08×10^{-6}	1.67×10^{-4}
Abamectin							1.55×10^{-5}	5.48×10^{-5}
Acrylamide					1.58×10^{-6}	1.21×10^{-4}		
Amcinonide							1.42×10^{-9}	5.98×10^{-1}
Benzophenone-3	2.26×10^{-6}[f]	1.07×10^{-6}						
Bethamethasone					1.92×10^{-10}	9.95×10^{-1}	5.06×10^{-10}	1.68×10^{0}
Bifonazol							1.27×10^{-5}	6.69×10^{-5}
Bis(2-ethylhexyl)phosphate							5.25×10^{-6}	1.62×10^{-4}
Bisphenol A	6.91×10^{-8}[e]	3.50×10^{-5}	4.50×10^{-7}	5.55×10^{-5}				
Bisphenol C			6.37×10^{-6}	3.92×10^{-6}				

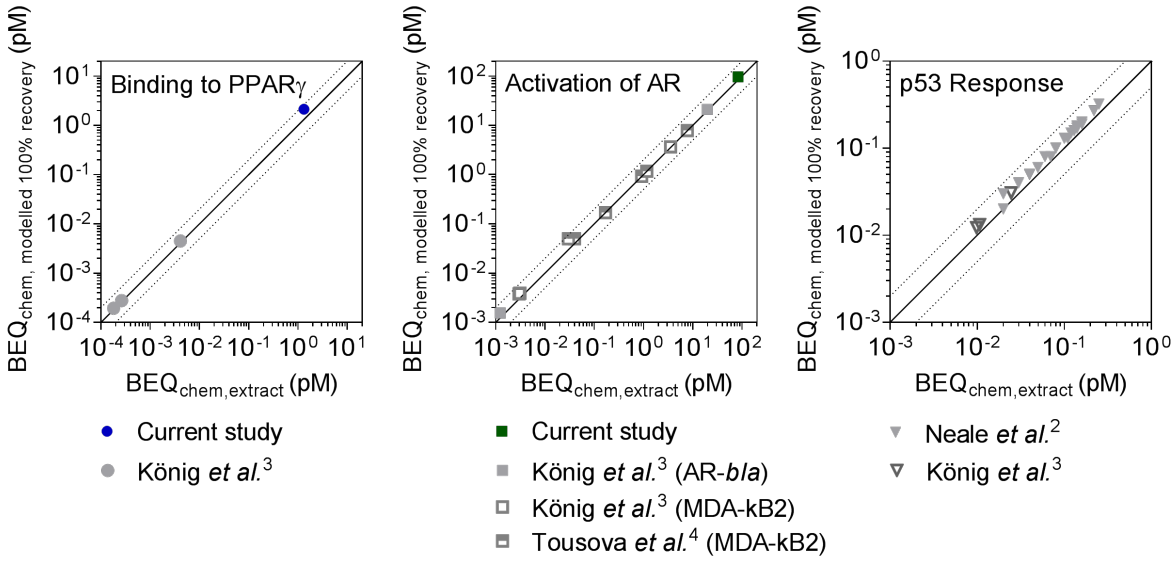
	MELN		ER GeneBLAzer		AR GeneBLAzer		GR GeneBLAzer	
	EC_{10} (M)	REP_i	EC_{10} (M)[j]	REP_i	EC_{10} (M)[j]	REP_i	EC_{10} (M)[j]	REP_i
Bisphenol E			8.26×10^{-7}	3.03×10^{-5}				
Bisphenol F	1.09×10^{-7}[g]	2.22×10^{-5}	2.98×10^{-6}	8.39×10^{-6}				
Bisphenol S	1.34×10^{-6}[g]	1.80×10^{-6}	1.60×10^{-6}	1.56×10^{-5}				
Bisphenol Z							6.82×10^{-6}	1.25×10^{-4}
Bromoxynil					1.25×10^{-6}	1.53×10^{-4}		
Budesonide							1.49×10^{-10}	5.70×10^{0}
Butylparaben	1.32×10^{-6}[h]	4.88×10^{-6}[h]	4.46×10^{-6}	5.60×10^{-6}				
Candesartan							1.86×10^{-6}	4.57×10^{-4}
Canrenone					1.09×10^{-7}	1.75×10^{-3}		
Celecoxib							2.99×10^{-6}	2.84×10^{-4}
Chlorophene							2.50×10^{-6}	3.40×10^{-4}
Clobetasol							1.07×10^{-10}	7.94×10^{0}
Clotrimazole					1.26×10^{-5}	1.52×10^{-5}	2.31×10^{-6}	3.68×10^{-4}
Cortisone					4.41×10^{-9}	4.33×10^{-2}		
Daidzein	2.45×10^{-8}[i]	9.86×10^{-5}	4.87×10^{-7}	5.13×10^{-5}				
DCOIT					4.42×10^{-6}	4.32×10^{-5}		
Desonide							2.54×10^{-9}	3.34×10^{-1}
Desoximetasone					3.69×10^{-7}	5.18×10^{-4}	9.05×10^{-10}	9.38×10^{-1}
Dexamethasone					1.67×10^{-7}	1.14×10^{-3}	8.49×10^{-10}[c]	1.00×10^{0}
Diazinon	8.53×10^{-6}[e]	2.83×10^{-7}						
Dichlorophen							3.35×10^{-6}	2.54×10^{-4}
Difenoconazole							1.00×10^{-5}	8.49×10^{-5}
Dihydrotestosterone			5.47×10^{-8}	4.56×10^{-4}	3.35×10^{-10}	5.70×10^{-1}		
Dodecylbenzenesulfonic acid							1.51×10^{-5}	5.62×10^{-5}

	MELN		ER GeneBLAzer		AR GeneBLAzer		GR GeneBLAzer	
	EC_{10} (M)	REP_i	EC_{10} (M)[j]	REP_i	EC_{10} (M)[j]	REP_i	EC_{10} (M)[j]	REP_i
Drospirenone					2.89×10^{-9}	6.61×10^{-2}		
Dydrogesterone					6.32×10^{-8}	3.02×10^{-3}	8.91×10^{-11}	9.53×10^{0}
Ebastin							1.40×10^{-6}	6.07×10^{-4}
Estriol	1.11×10^{-11}[b]	2.17×10^{-1}	3.82×10^{-10}	6.55×10^{-2}				
Estrone	2.26×10^{-11}[e]	1.07×10^{-1}	1.12×10^{-10}	2.24×10^{-1}				
Exemestane					3.29×10^{-8}	5.81×10^{-3}		
Flunisolide							8.49×10^{-10}	1.00×10^{0}
Fluorometholone					1.96×10^{-10}	9.75×10^{-1}	1.81×10^{-10}	4.69×10^{0}
Fluticasone propionate							3.00×10^{-11}	2.83×10^{1}
Gemfibrozil		4.70×10^{-8}[d]						
Genistein	1.22×10^{-8}[e]	1.98×10^{-4}	8.57×10^{-7}	2.91×10^{-4}				
Gestoden			7.96×10^{-8}	3.14×10^{-4}	1.55×10^{-10}	1.23×10^{0}		
Hydrocortisonacetate					4.64×10^{-9}	4.12×10^{-2}	2.16×10^{-8}	3.93×10^{-2}
Hydrocortisone					1.20×10^{-9}	1.59×10^{-1}	4.82×10^{-9}	1.76×10^{-1}
Imazalil			3.30×10^{-5}	7.56×10^{-7}	8.34×10^{-6}	2.29×10^{-5}	2.04×10^{-5}	4.16×10^{-5}
Iopamidol							1.39×10^{-6}	6.11×10^{-4}
Ketoconazole			2.26×10^{-5}	1.11×10^{-6}	7.31×10^{-6}	2.61×10^{-5}	8.32×10^{-6}	1.02×10^{-4}
Medroxyprogesterone					6.83×10^{-11}	2.80×10^{0}	3.44×10^{-8}	2.47×10^{-2}
Medroxyprogesteroneacetate					3.53×10^{-8}	5.41×10^{-3}	1.32×10^{-8}	6.43×10^{-2}
Mestranol	5.90×10^{-10}[h]	2.00×10^{-2}[h]	7.69×10^{-9}	3.25×10^{-3}				
Methylchloroisothiazolinone							2.32×10^{-5}	3.66×10^{-5}
Musk ambrette			2.03×10^{-5}	1.23×10^{-6}				
Norethindrone			4.91×10^{-8}	5.09×10^{-4}	4.84×10^{-10}	3.95×10^{-1}		
Norgestimate			1.30×10^{-7}	1.92×10^{-4}	8.14×10^{-9}	2.35×10^{-2}	3.68×10^{-6}	2.31×10^{-4}

	MELN		ER GeneBLAzer		AR GeneBLAzer		GR GeneBLAzer	
	EC_{10} (M)	REP_i	EC_{10} (M)[j]	REP_i	EC_{10} (M)[j]	REP_i	EC_{10} (M)[j]	REP_i
Norgestrel			3.77×10^{-7}	6.63×10^{-5}	5.22×10^{-10}	3.66×10^{-1}		
Oryzalin							5.42×10^{-6}	1.57×10^{-4}
Perfluorooctanesulfonamide			2.43×10^{-5}	1.03×10^{-6}				
Prednisolone					3.83×10^{-9}	4.99×10^{-2}	2.47×10^{-9}	3.44×10^{-1}
Prednisone					6.99×10^{-9}	2.73×10^{-2}		
Pregnanediol			2.11×10^{-6}	1.19×10^{-5}				
Progesterone		7.50×10^{-7}[d]			2.35×10^{-9}	8.13×10^{-2}		
Promethazin			3.51×10^{-6}	7.12×10^{-6}	2.12×10^{-6}	9.01×10^{-5}	3.12×10^{-6}	2.72×10^{-4}
Propylparaben	7.27×10^{-7}[h]	7.40×10^{-6}[h]	9.15×10^{-6}	2.73×10^{-6}				
Raloxifene							1.32×10^{-5}	6.43×10^{-5}
Tamoxifen			4.79×10^{-6}	5.22×10^{-6}	3.40×10^{-6}	5.62×10^{-5}	3.69×10^{-6}	2.30×10^{-4}
Terbuthylazine	1.52×10^{-5}[e]	1.59×10^{-7}	8.47×10^{-6}	2.95×10^{-6}				
Testosterone			1.60×10^{-6}	1.56×10^{-5}	9.27×10^{-11}	2.06×10^{0}		
Tetrabromobisphenol A							1.14×10^{-5}	7.45×10^{-5}
Trenbolone			1.10×10^{-7}	2.27×10^{-4}	1.45×10^{-10}	1.32×10^{0}		
Triamcinolone					1.30×10^{-5}	1.47×10^{-5}	4.15×10^{-9}	$2.05\times10^{--1}$
Triclosan					1.08×10^{-5}	1.77×10^{-5}	2.54×10^{-6}	3.34×10^{-4}
Triphenylphosphate	1.71×10^{-6}[e]	1.41×10^{-6}						
Ziprasidone					5.14×10^{-6}	3.72×10^{-5}	5.84×10^{-6}	1.45×10^{-4}

[a]Creusot *et al.*[16]; [b]Pillon *et al.*[17]; [c]Current study; [d]Creusot *et al.*[18]; [e]Neale *et al.*[11]; [f]Molina-Molina *et al.*[19]; [g]Molina-Molina *et al.*[20]; [h]Kinani *et al.*[21]; [i]Neale *et al.*[2]; [j]US EPA[14]

Figure S13: Comparison of $BEQ_{chem,extract}$ and $BEQ_{chem,\ modelled\ 100\%\ recovery}$ for binding to PPARγ, activation of AR and p53 response derived from LVSPE recovery data (Table S5), with data from the current study, Neale *et al.*[2], König *et al.*[3] and Tousova *et al.*[4] The dotted lines indicate a factor of 2 difference between $BEQ_{chem,extract}$ and $BEQ_{chem,\ modelled\ 100\%\ recovery}$.



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