Supporting Information

Influence of Co-Dosed Lipids from Biota Extracts on the Availability of Chemicals in *in vitro* Cell-Based Bioassays

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Table of Content

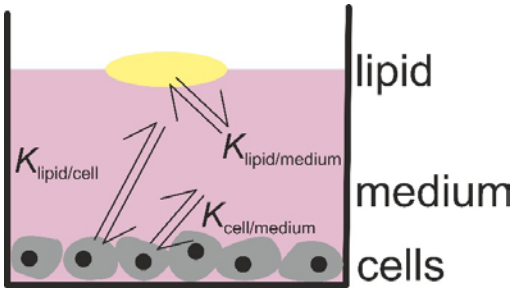


Figure SI 1. Schematic illustration of the three-phase system used for the present study. The chemical partitioning is described by the partition constants *K* between lipid, medium and cells. In this simplified model cell and medium components composed of water, proteins and lipids are integrated into one system parameter referred to as 'cell' and 'medium', respectively.

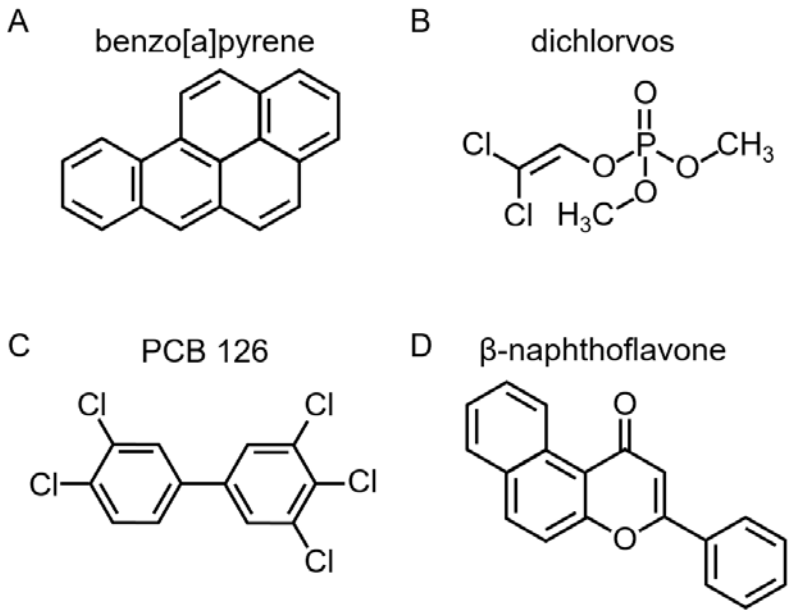


Figure SI 2. Chemical structures of the four test chemicals: **A.** benzo[a]pyrene, **B.** dichlorvos, **C.** PCB 126 and **D.** β-naphthoflavone.

Section SI 1. Additional information on the volume fractions of medium and cell components in the reporter gene cell lines and the cell numbers of the plated cells

AREc32 and AhR-CALUX cells were tested in 90% DMEM with Glutamax and 10% FBS. The volume fractions V*f* of the medium components water, protein and lipid are: Vf_{water} = 99.09, $Vf_{protein}$ = 0.893 % and Vf_{lipid} = 0.0139 %.[1]

The volume fractions V*f* in the cell compartments water, protein and lipid are listed in Table SI 1.

Table SI 1. Evaluated number of plated cells and their average during the experiment in the 384-well plates for the two different reporter gene cell lines. Additionally, the volume fractions of the cell components water, protein and lipid are given.

reporter gene cell line	number of plated cells/well	growth ratio (0 – 48 h)	number of cells in the assay	$Vf_{water,cell}$[a]	$Vf_{protein,cell}$[a]	$Vf_{lipid,cell}$[a]
AREc32	2500	2.3 ± 0.2	4110 ± 280	94.4%	5.14%	0.463%
AhR-CALUX	3250	3.3 ± 0.5	6970 ± 780	93.9%	5.49%	0.580%

[a]Henneberger et al.[2]

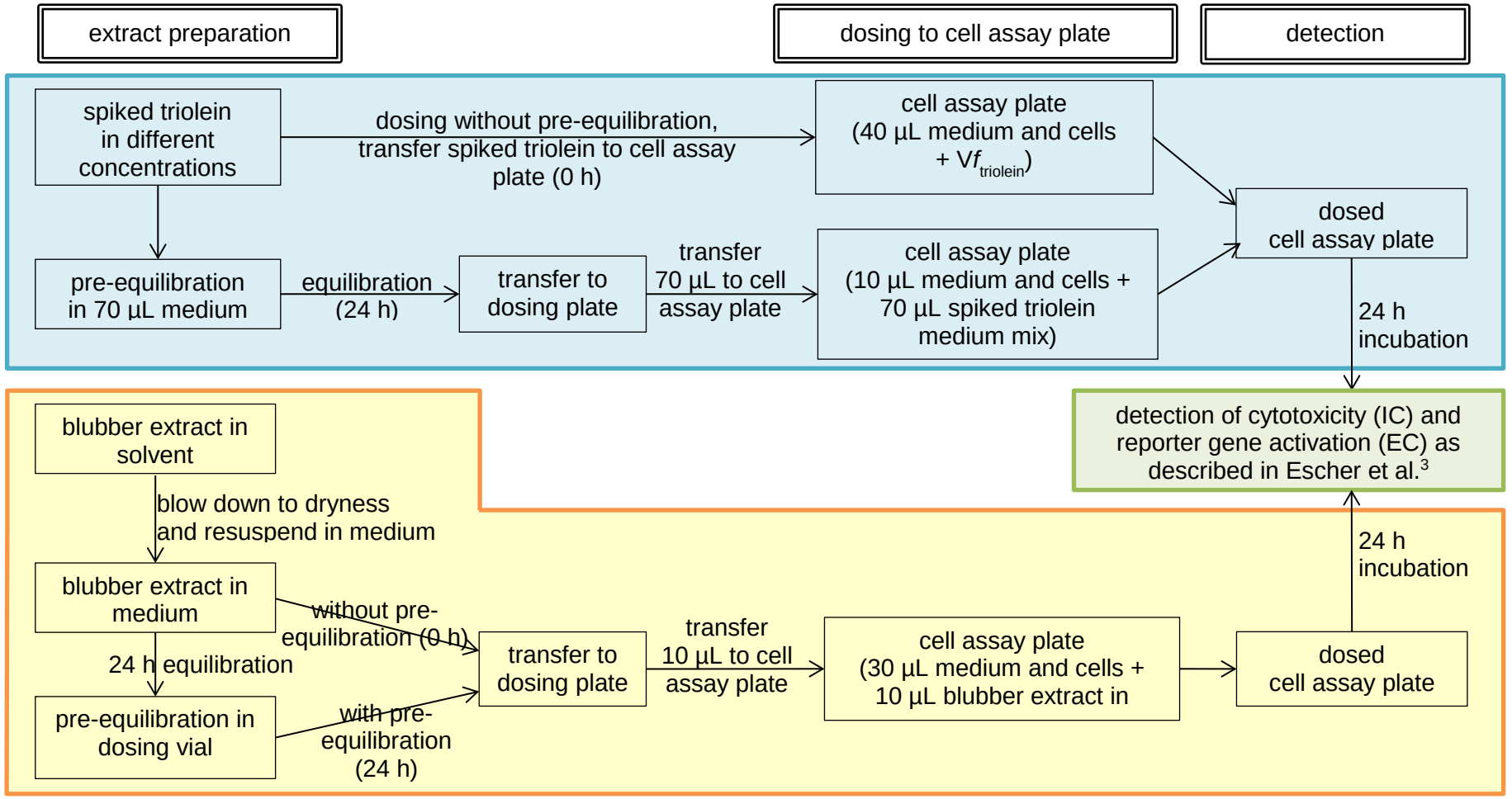


Figure SI 3. Workflow of the dosing procedure for spiked triolein (blue box) and blubber extracts sampled with PDMS (yellow box) to the bioassays.

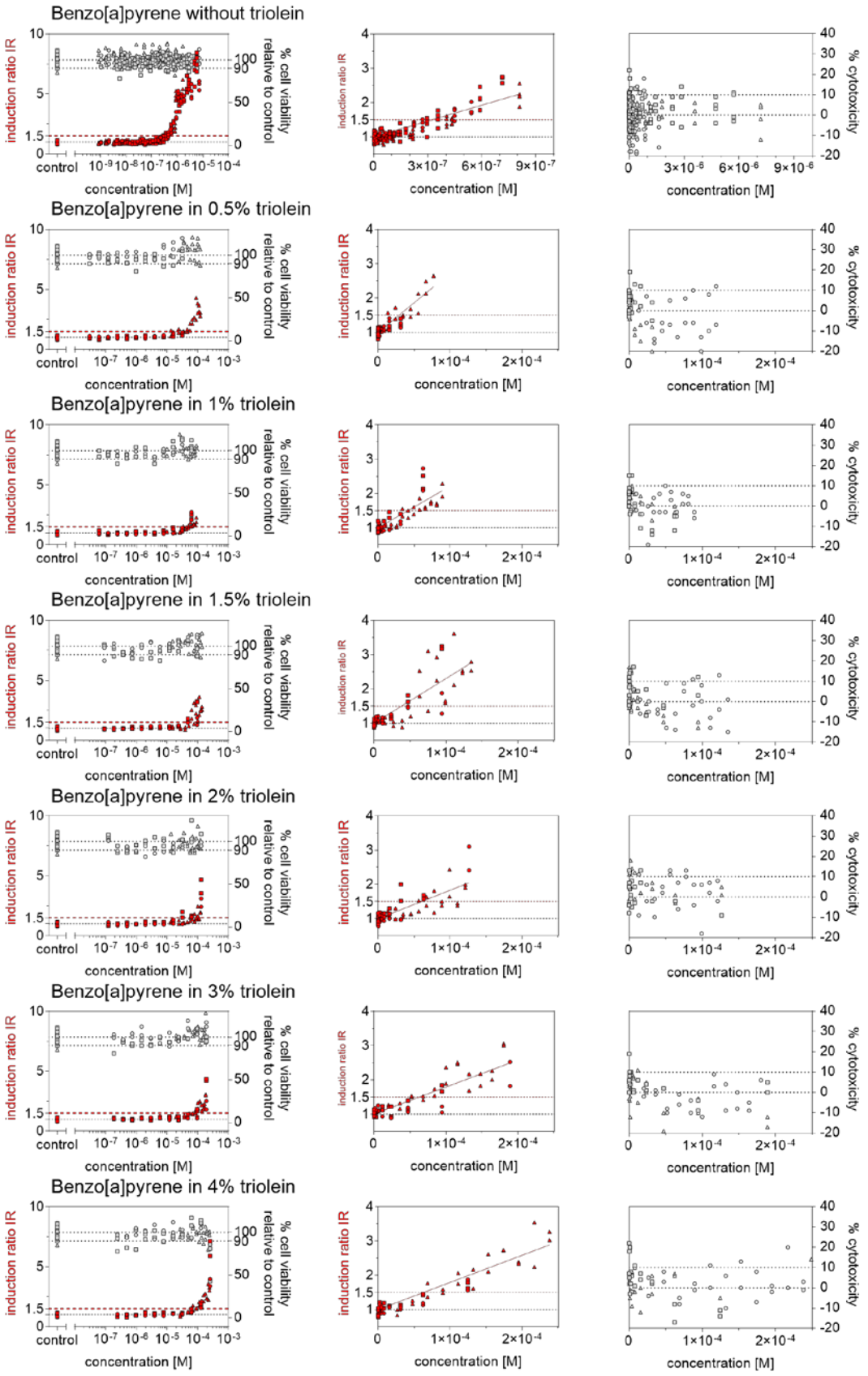


Figure SI 4. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for Benzo[a]pyrene in AREc32 without pre-equilibration prior to dosing (0 h). All concentrations are nominal concentrations.

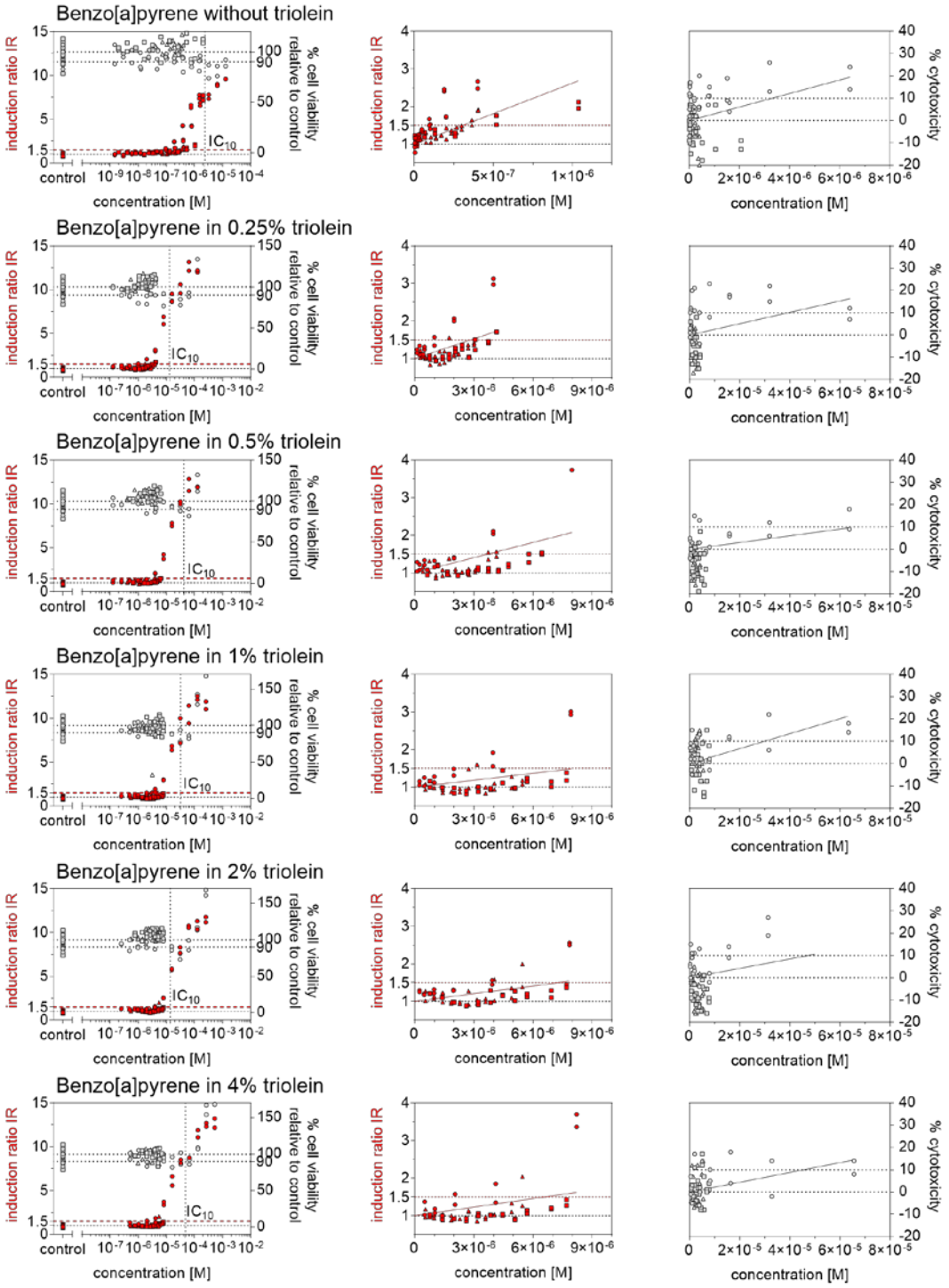


Figure SI 5. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for Benzo[a]pyrene in AREc32 with 24h pre-equilibration prior to dosing (24 h). All concentrations are nominal concentrations.

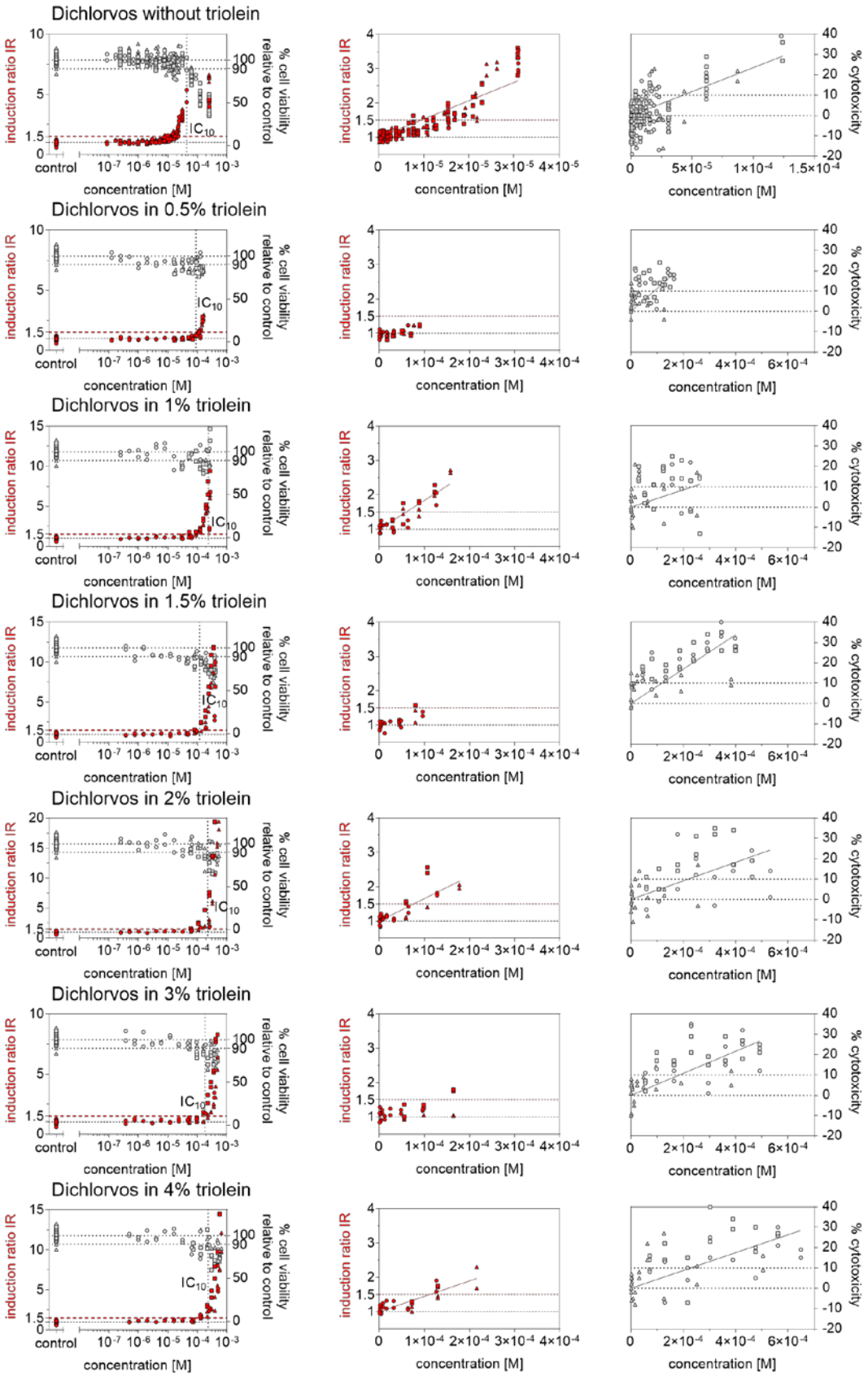


Figure SI 6. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for Dichlorvos in AREc32 without pre-equilibration prior to dosing (0 h). All concentrations are nominal concentrations.

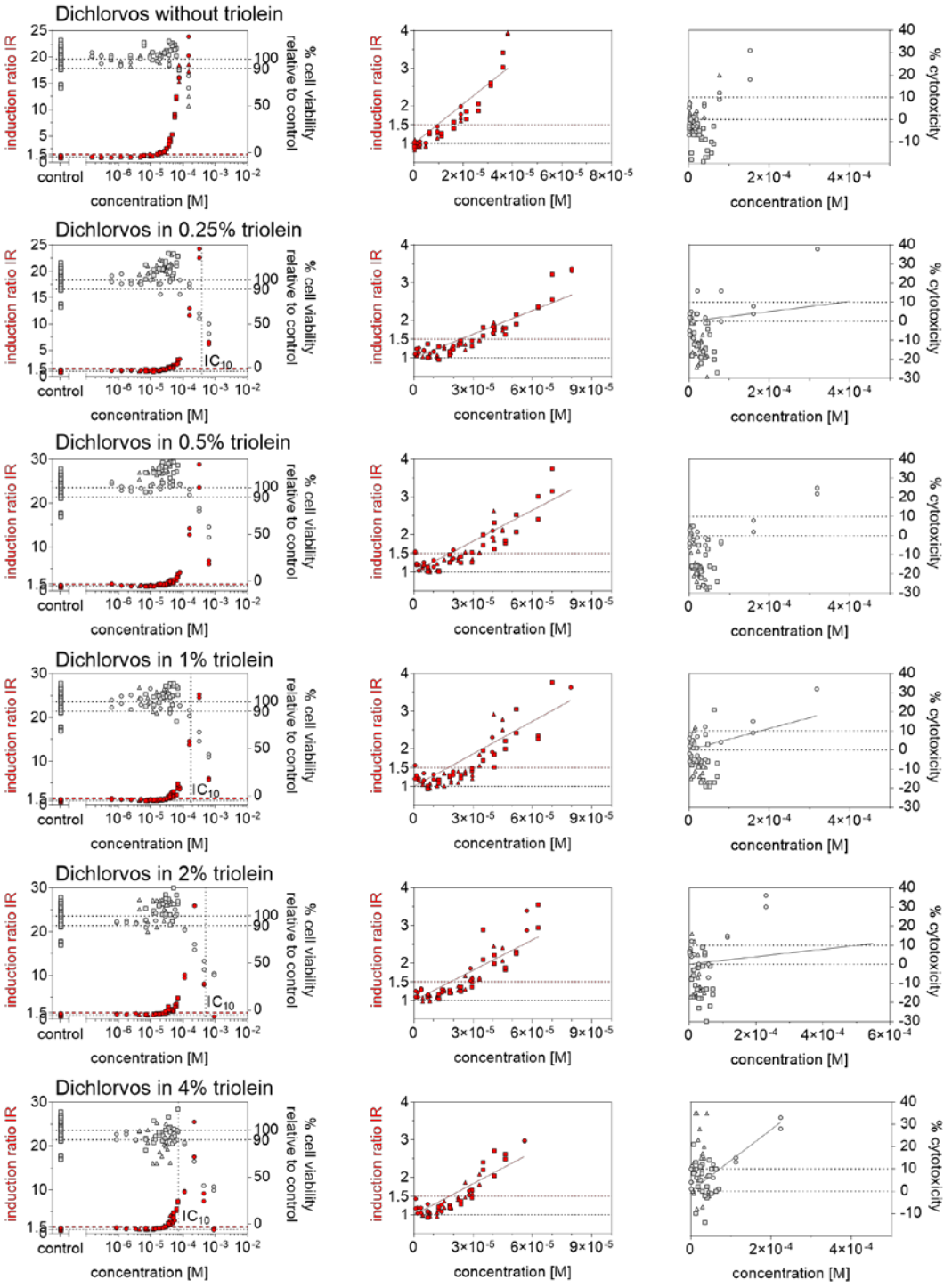


Figure SI 7. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for Dichlorvos in AREc32 with 24h pre-equilibration prior to dosing (24 h). All concentrations are nominal concentrations.

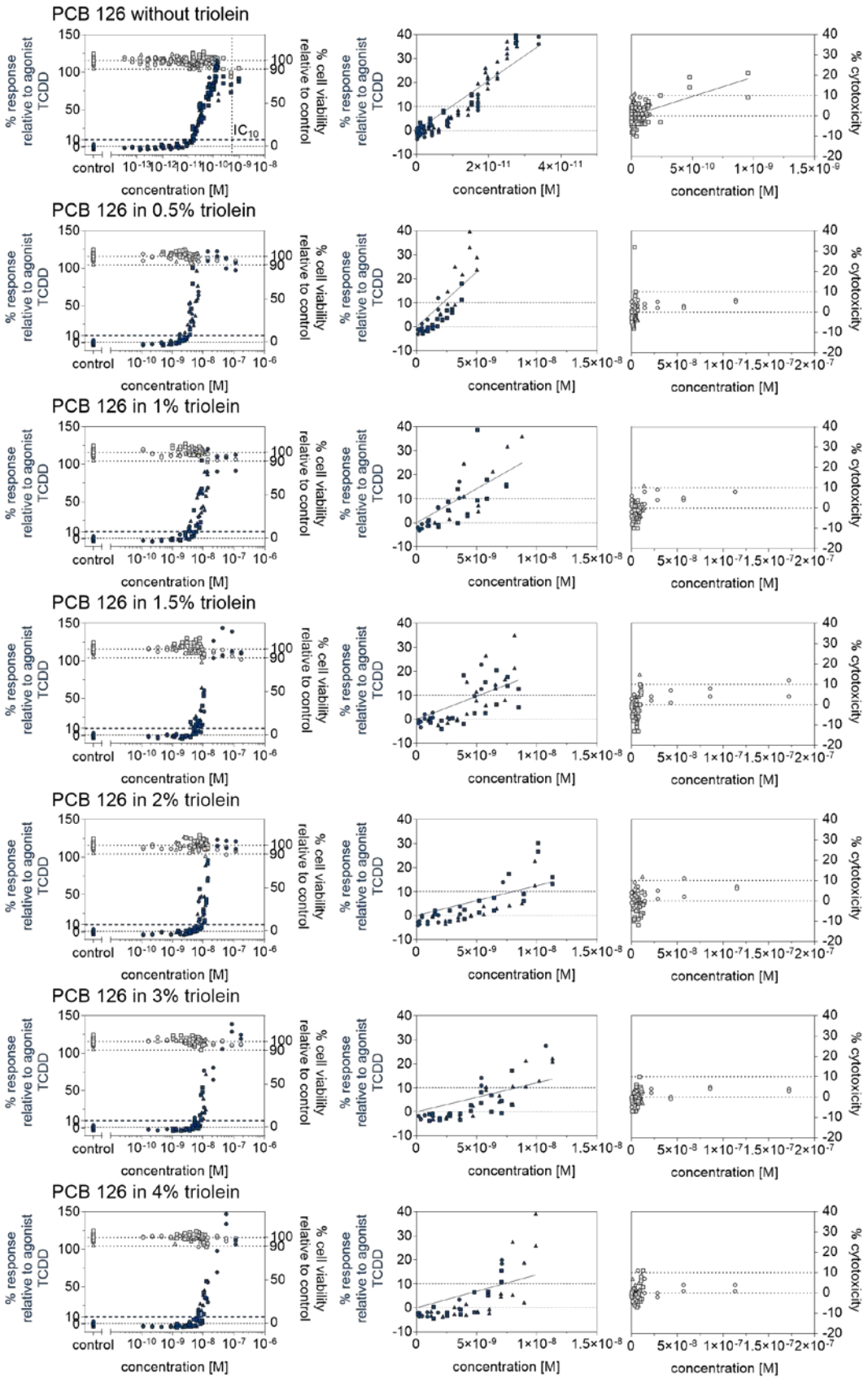


Figure SI 8. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for PCB 126 in AhR-CALUX without pre-equilibration prior to dosing (0 h). All concentrations are nominal concentrations.

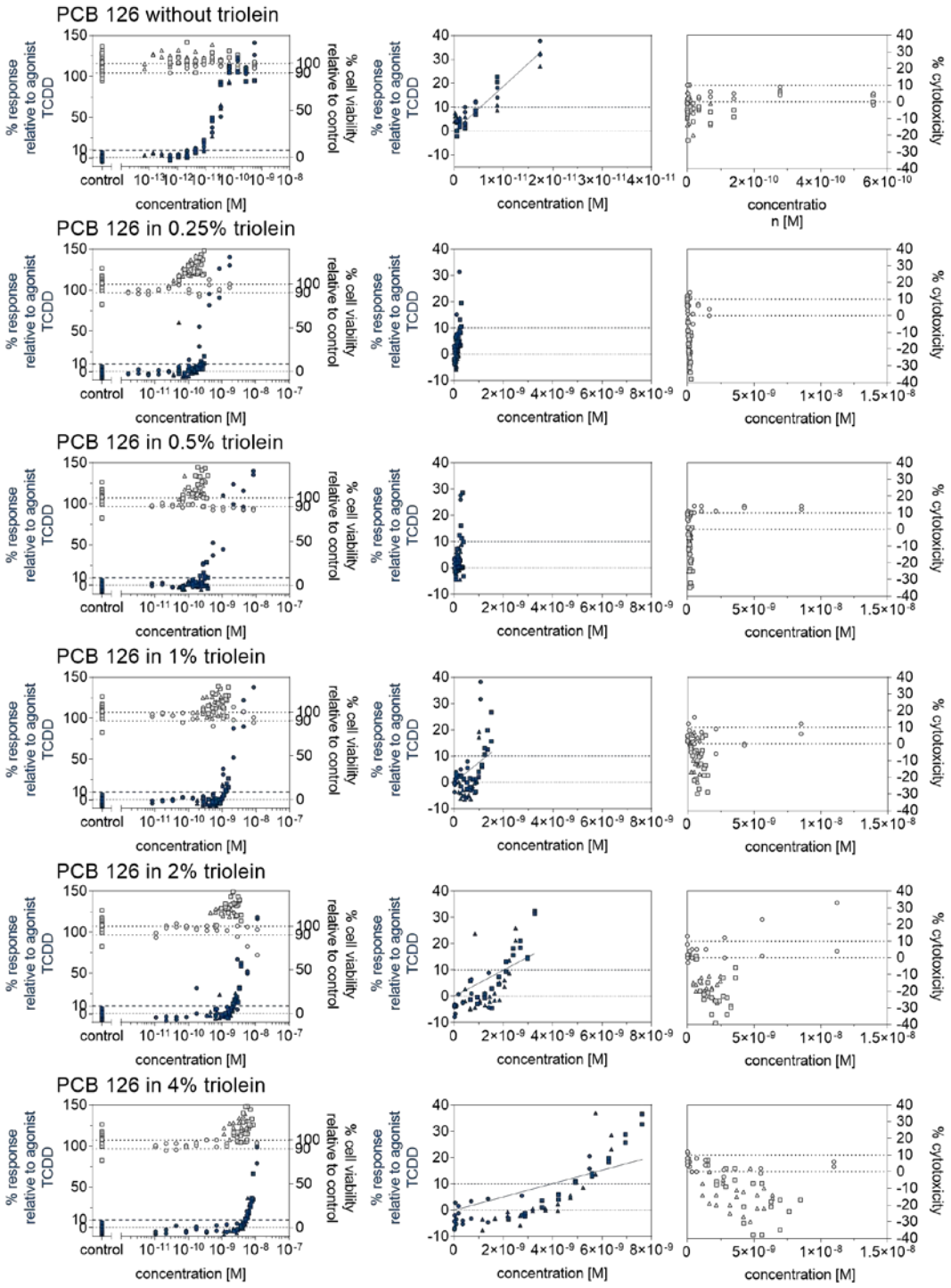


Figure SI 9. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for PCB 126 in AhR-CALUX with 24h pre-equilibration prior to dosing (24 h). All concentrations are nominal concentrations.

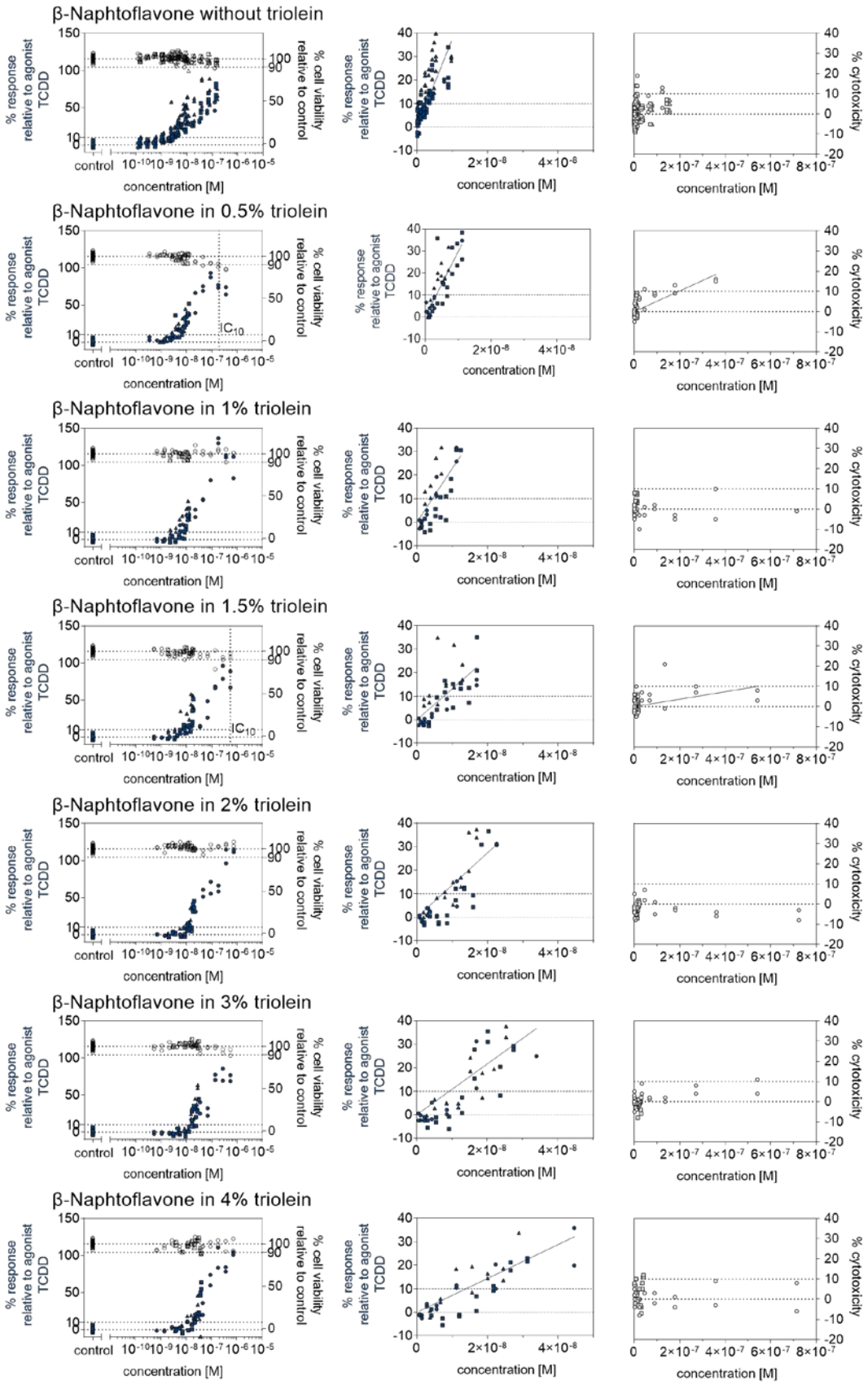


Figure SI 10. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for β-naphthoflavone in AhR-CALUX without pre-equilibration prior to dosing (0 h). All concentrations are nominal concentrations.

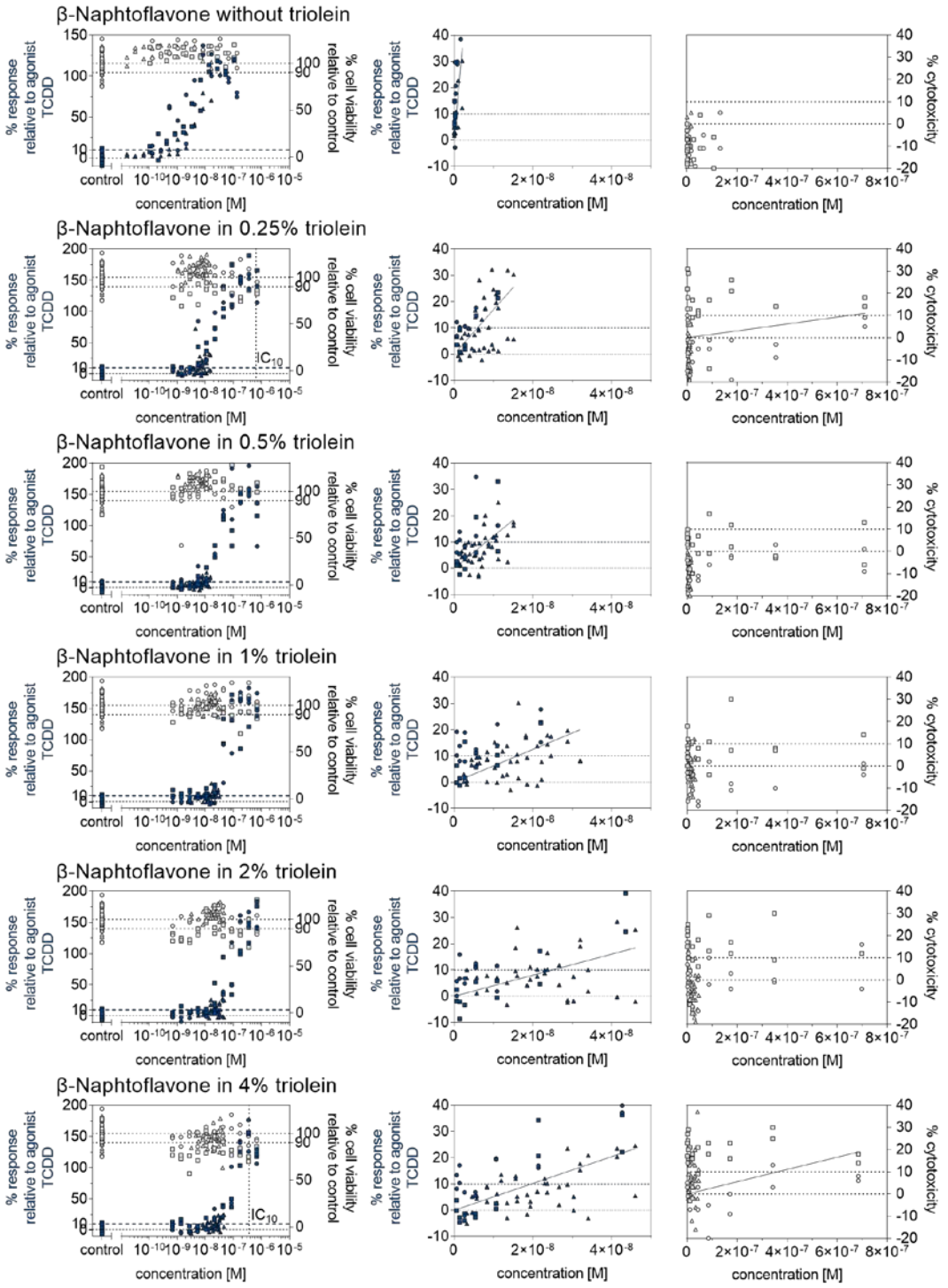


Figure SI 11. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for β-naphthoflavone in AhR-CALUX with 24h pre-equilibration prior to dosing (24 h). All concentrations are nominal concentrations.

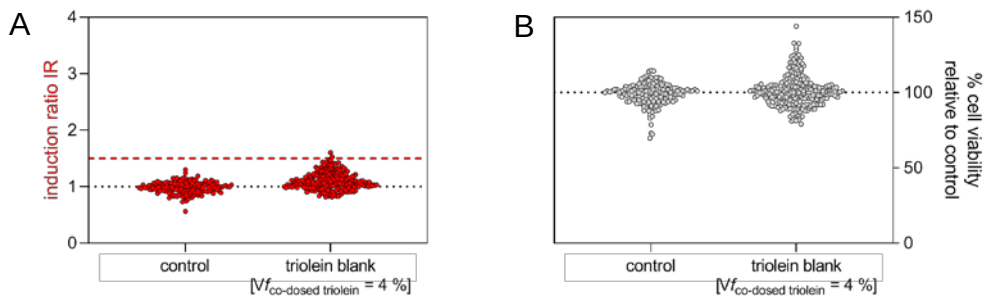


Figure SI 12. Induction ratio IR (A) and the cell viability (B) of triolein blanks in the AREc32 assay for a V$f_{\text{co-dosed triolein}}$ of 4 %, which was the highest amount of co-dosed triolein.

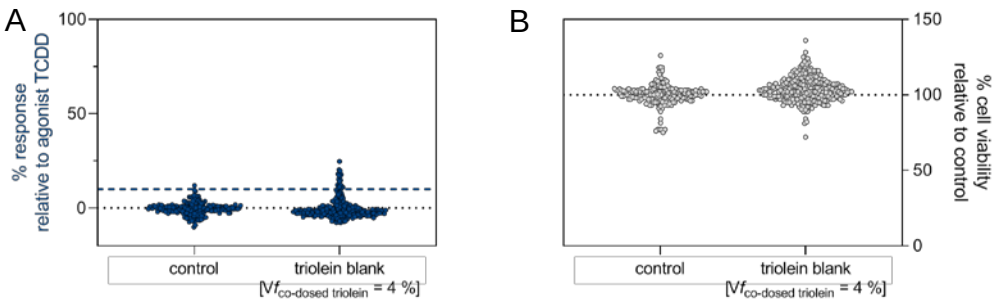


Figure SI 13. Response relative to the agonist TCDD (A) and the cell viability (B) of triolein blanks in the AhR-CALUX assay for a V$f_{\text{co-dosed triolein}}$ of 4 %, which was the highest amount of co-dosed triolein.

Table SI 2. Effect concentrations $EC_{IR1.5}$ and EC_{10} and inhibitory concentrations IC_{10} for cytotoxicity from experiments with spiked triolein for '0 h' and '24 h' for different Volume fractions V*f* of triolein. Mean EC and IC and their associated standard errors (SE) that are derived from the linear regression of the concentration-effect relationships are shown in Figures SI 3 to SI-10.

'0 h'	Benzo[a]pyrene		Dichlorvos		PCB 126		β-Naphthoflavone	
V*f* triolein [%]	$EC_{IR1.5}$ [M]	IC_{10} [M]	$EC_{IR1.5}$ [M]	IC_{10} [M]	EC_{10} [M]	IC_{10} [M]	EC_{10} [M]	IC_{10} [M]
0	$(3.3\pm0.1)\cdot10^{-7}$	$> 4.7\cdot10^{-6}$	$(9.4\pm0.3)\cdot10^{-6}$	$(4.3\pm0.3)\cdot10^{-5}$	$(9.6\pm0.3\cdot10^{-12}$	$(5.2\pm0.7)\cdot10^{-10}$	$(2.7\pm0.1)\cdot10^{-9}$	$> 1.6\cdot10^{-7}$
0.5	$(2.9\pm0.1)\cdot10^{-5}$	$> 1.2\cdot10^{-4}$	$> IC_{10}$	$(8.9\pm1.0)\cdot10^{-5}$	$(2.2\pm0.2)\cdot10^{-9}$	$> 1.1\cdot10^{-7}$	$(3.4\pm0.2)\cdot10^{-9}$	$(2.0\pm0.2)\cdot10^{-7}$
1.0	$(4.1\pm0.3)\cdot10^{-5}$	$> 8.9\cdot10^{-5}$	$(6.0\pm0.3)\cdot10^{-5}$	$(2.4\pm0.6)\cdot10^{-4}$	$(3.5\pm0.4)\cdot10^{-9}$	$>1.1\cdot10^{-7}$	$(4.4\pm0.4)\cdot10^{-9}$	$> 7.1\cdot10^{-7}$
1.5	$(3.8\pm0.3)\cdot10^{-5}$	$> 1.4\cdot10^{-4}$	$> IC_{10}$	$(1.2\pm0.1)\cdot10^{-4}$	$(5.2\pm0.5)\cdot10^{-9}$	$> 1.7\cdot10^{-7}$	$(7.6\pm0.7)\cdot10^{-9}$	$(5.4\pm1.4)\cdot10^{-7}$
2.0	$(6.0\pm0.4)\cdot10^{-5}$	$> 1.3\cdot10^{-4}$	$(7.6\pm0.8)\cdot10^{-5}$	$(2.2\pm0.3)\cdot10^{-4}$	$(8.0\pm0.9)\cdot10^{-9}$	$> 1.2\cdot10^{-7}$	$(7.3\pm0.6)\cdot10^{-9}$	$> 7.2\cdot10^{-7}$
3.0	$(6.3\pm0.3)\cdot10^{-5}$	$> 1.9\cdot10^{-4}$	$> IC_{10}$	$(1.8\pm0.2)\cdot10^{-4}$	$(8.3\pm1.0)\cdot10^{-9}$	$> 1.7\cdot10^{-7}$	$(9.3\pm0.6)\cdot10^{-9}$	$> 5.4\cdot10^{-7}$
4.0	$(6.3\pm0.2)\cdot10^{-5}$	$> 2.5\cdot10^{-4}$	$(1.1\pm0.1)\cdot10^{-4}$	$(2.3\pm0.2)\cdot10^{-4}$	$(7.3\pm1.2)\cdot10^{-9}$	$> 1.1\cdot10^{-7}$	$(1.4\pm0.1)\cdot10^{-8}$	$> 7.1\cdot10^{-7}$
'24 h'	Benzo[a]pyrene		Dichlorvos		PCB 126		β-Naphthoflavone	
V*f* triolein [%]	$EC_{IR1.5}$ [M]	IC_{10} [M]	$EC_{IR1.5}$ [M]	IC_{10} [M]	EC_{10} [M]	IC_{10} [M]	EC_{10} [M]	IC_{10} [M]
0	$(3.1\pm0.3)\cdot10^{-7}$	$(3.3\pm0.9)\cdot10^{-6}$	$(9.5\pm0.5)\cdot10^{-6}$	$> 1.5\cdot10^{-4}$	$(5.3\pm0.3)\cdot10^{-12}$	$> 5.6\cdot10^{-10}$	$(8.2\pm1.0)\cdot10^{-10}$	$> 1.3\cdot10^{-7}$
0.25	$(2.9\pm0.4)\cdot10^{-6}$	$(3.9\pm1.4)\cdot10^{-5}$	$(2.4\pm0.1)\cdot10^{-5}$	$(3.9\pm4.12)\cdot10^{-4}$	$(9.6\pm1.5)\cdot10^{-11}$	$> 1.7\cdot10^{-9}$	$(5.9\pm0.8)\cdot10^{-9}$	$(6.4\pm3.2)\cdot10^{-7}$
0.50	$(3.7\pm0.5)\cdot10^{-6}$	$(6.6\pm3.5)\cdot10^{-5}$	$(1.8\pm0.1)\cdot10^{-5}$	$> 6.4\cdot10^{-4}$	$(1.3\pm0.2)\cdot10^{-10}$	$> 4.5\cdot10^{-9}$	$(8.2\pm0.8)\cdot10^{-9}$	$> 7.1\cdot10^{-7}$
1.00	$(8.2\pm1.7)\cdot10^{-6}$	$(3.0\pm0.9)\cdot10^{-5}$	$(1.7\pm0.1)\cdot10^{-5}$	$(1.8\pm0.6)\cdot10^{-4}$	$(3.6\pm0.3)\cdot10^{-10}$	$> 8.5\cdot10^{-9}$	$(1.6\pm0.2)\cdot10^{-8}$	$> 7.1\cdot10^{-7}$
2.00	$(7.3\pm1.1)\cdot10^{-6}$	$(4.7\pm3.9)\cdot10^{-5}$	$(1.9\pm0.1)\cdot10^{-5}$	$(5.1\pm8.4)\cdot10^{-4}$	$(2.01\pm0.2)\cdot10^{-9}$	$> 4.9\cdot10^{-9}$	$(2.5\pm0.3)\cdot10^{-8}$	$> 7.0\cdot10^{-7}$
4.00	$(6.6\pm1.4)\cdot10^{-6}$	$(4.5\pm1.3)\cdot10^{-5}$	$(1.8\pm0.1)\cdot10^{-5}$	$(7.2\pm1.4)\cdot10^{-5}$	$(3.9\pm0.8)\cdot10^{-9}$	$> 1.1\cdot10^{-8}$	$(2.0\pm0.2)\cdot10^{-8}$	$(3.6\pm1.1)\cdot10^{-7}$

Section SI 2. Information on the partition constants *K* for the three-phase partitioning model

If no literature data for $K_{medium/w}$ and $K_{cell/w}$ were available, the partition constants were calculated with equations SI 1 and SI 2 using bovine serum albumin (BSA) as a surrogate for proteins in cell and medium ($K_{BSA/w}$) and the liposome/water partition constant $K_{lip/w}$ was used as a surrogate for lipids in cells and medium.

$$K_{medium/w} = \frac{V_{protein,medium}}{V_{medium}} \cdot K_{BSA/w} + \frac{V_{lipid,medium}}{V_{medium}} \cdot K_{lipid/w} + \frac{V_{water,medium}}{V_{medium}} \quad \text{(SI 1)}$$

$$K_{cell/w} = \frac{V_{protein,cell}}{V_{cell}} \cdot K_{BSA/w} + \frac{V_{lipid,cell}}{V_{cell}} \cdot K_{lipid/w} + \frac{V_{water,cell}}{V_{cell}} \quad \text{(SI 2)}$$

For chemicals where no $K_{BSA/w}$, $K_{lip/w}$ and $K_{triolein/w}$ were available, these partition constants were predicted with polyparameter linear-solvation energy relationships (PP-LFER), shown in equation SI 3 from Endo et al. ,[4] equation SI 4 from Endo et al.[5] and equation SI 5 from Abraham et al.[6] to estimate $K_{BSA/w}$, $K_{lip/w}$, and $K_{triolein/w}$.

$$\log K_{BSA/w} = 0.35 + 0.28 \cdot L - 0.46 \cdot S + 0.2 \cdot A - 3.18 \cdot B + 1.84 \cdot V \quad \text{(SI 3)}$$

$$\log K_{lip/w} = 0.53 + 0.49 \cdot L - 0.93 \cdot S + 0.18 \cdot A - 3.75 \cdot B + 1.73 \cdot V \quad \text{(SI 4)}$$

$$\log K_{triolein/w} = 0.531 + 3.522 \cdot V - 1.191 \cdot S - 3.110 \cdot A - 3.913 \cdot B + 0.856 \cdot E \quad \text{(SI 5)}$$

The parameters described by the logarithm of the hexadecane-air partition constant (L), polarizability/dipolarity parameter (S), effective hydrogen H-bond acidity (A) and basicity (B), molar volume (V) and excess molar refraction (E) were taken from the UFZ-LSER database[7] (Table SI 3).

For evaluation of the general hydrophobicity dependence, simple linear models were applied to calculate log $K_{BSA/w}$ and log $K_{lip/w}$ from the log K_{ow} with equation SI 6 from Endo et al.[4] and equation SI 7 from Endo et al.[5]

$$\log K_{BSA/w} = 0.71 \cdot \log K_{ow} + 0.42 \quad \text{(SI 6)}$$

$$\log K_{lip/w} = 1.01 \cdot \log K_{ow} + 0.12 \quad \text{(SI 7)}$$

Table SI 3. Partition constants (*K*) of the four test chemicals between octanol/water, BSA/water, liposome/water, triolein/water, medium/water and cell/water.

Chemical name	log K_{ow}	log $K_{BSA/w}$	log $K_{lip/w}$	log $K_{triolein/w}$	log $K_{medium/w}$	log $K_{cell/w}$
benzo[a]pyrene	5.98[a]	4.98[b]	7.05[c]	6.44[d]	3.27[b]	4.76[b]
dichlorvos	1.45[e]	2.52[f]	2.66[g]	2.46[d]	0.83[h]	1.55[i]
PCB 126	7.05[j]	5.67[f]	6.74[k]	6.93[k]	3.80[h]	4.84[i]
β-naphthoflavone	4.78[l]	4.29[f]	5.39[g]	4.52[d]	2.24[h]	3.20[i]

[a]Miller et al.[8]; [b]Henneberger et al.[1]; [c]Van der Heijden et al.[9]; [d]equation SI 5[6]; [e]Kawamoto and Urano[10]; [f]calculated with equation SI 3[4]; [g]equation SI 4[5]; [h]equation SI 6; [i]equation SI 7; [j]Sabljic et al.[11]; [k]Quinn et al.[12]; [l]estimated with OPERA (CompTox Chemistry Dashboard).[13]

Table SI 4. Detailed descriptors for polyparameter linear-solvation energy relationships (PP-LFER) prediction for benzo[a]pyrene, dichlorvos, PCB 126 and β-naphthoflavone from the UFZ LSER database.[7]

Name	Estimated SMILES	L	S	A	B	V	E	Literature
benzo[a]pyrene	c1ccc2c(c1)c1ccc3c4c1c(c2)ccc4ccc3	11.74	1.98	0.00	0.44	1.95	3.63	LSER Dataset for CompTox users (2017)
dichlorvos	COP(=O)(OC=C(Cl)Cl)OC	4.84	1.61	0.00	0.27	1.31	0.32	LSER Dataset for CompTox users (2017)
PCB 126	Clc1ccc(cc1Cl)c1cc(Cl)c(c(c1)Cl)Cl	9.88	1.57	0.00	0.09	1.94	2.11	LSER Dataset for CompTox users (2017)
β-naphthoflavone	O=c1cc(oc2c1c1ccccc1cc2)c1ccccc1	11.58	1.91	0.12	0.69	2.04	2.52	calculated descriptors by using SMILES input

Section SI 3. Sensitivity analysis of the three-phase partitioning model

To estimate the uncertainties from the main variable factors $K_{BSA/w}$, $K_{lip/w}$ and $K_{triolein/w}$ of the three-phase model (equation 7 in the main text), we conducted a common local sensitivity analysis as a robustness check. As $K_{medium/w}$ and $K_{cell/w}$ were predicted with the $K_{BSA/w}$ and $K_{lip/w}$ (equation SI 1 and SI 2), these constants were not included to the sensitivity analysis. One of the partition constants K in Table 1 were increased and decreased by 10 %, 30 % and 50 % (ΔK), while the others were kept unchanged, resulting in changes of the modeled EC (ΔEC_{nom}). The relative change of these parameters (model elasticity) was calculated with equation SI 8, averaged over all scenarios and test chemicals and is depicted in Figure SI 14.

$$\text{model elasticity} = \frac{\Delta EC_{nom}}{EC_{nom}} \cdot \left(\frac{\Delta K}{K}\right)^{-1} \qquad \text{(SI 8)}$$

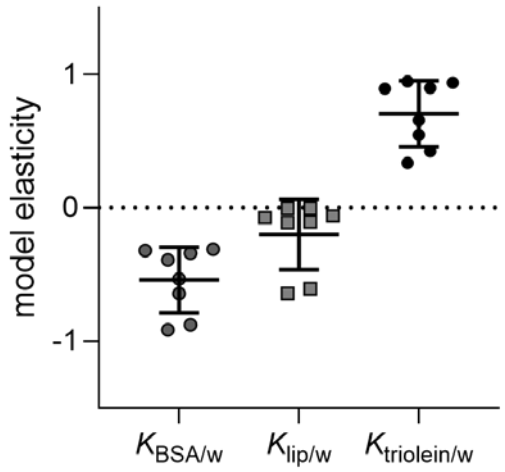
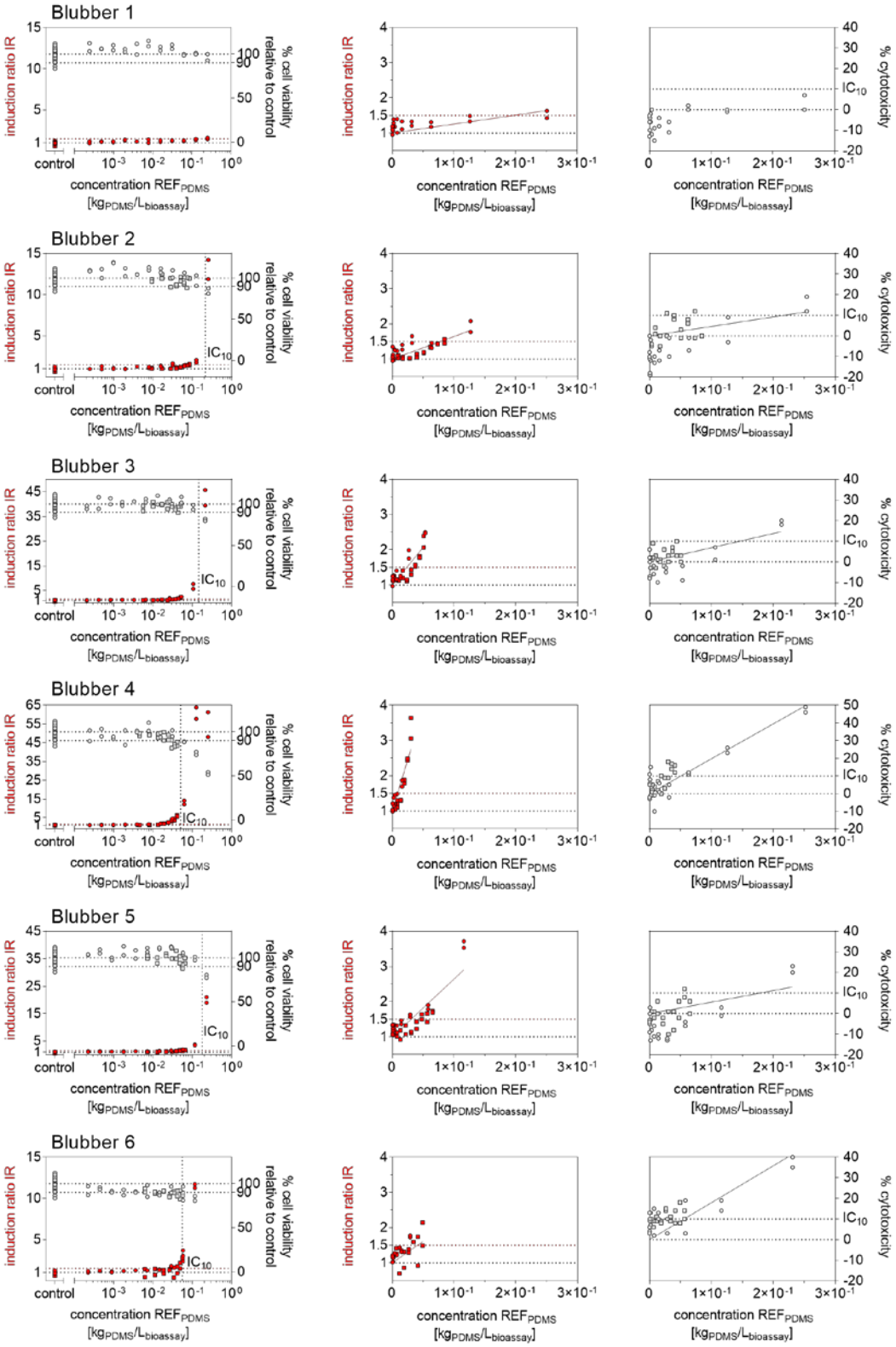


Figure SI 14. Model elasticity of the partition constants K used to calculate the modeled EC ($K_{BSA/w}$, $K_{medium/w}$ and $K_{triolein/w}$), calculated with equation SI 8.


Blubber 1
Blubber 2
Blubber 3
Blubber 4
Blubber 5
Blubber 6
induction ratio IR
% cell viability relative to control
% cytotoxicity
IC10
control
concentration REFPDMS
[kgPDMS/Lbioassay]

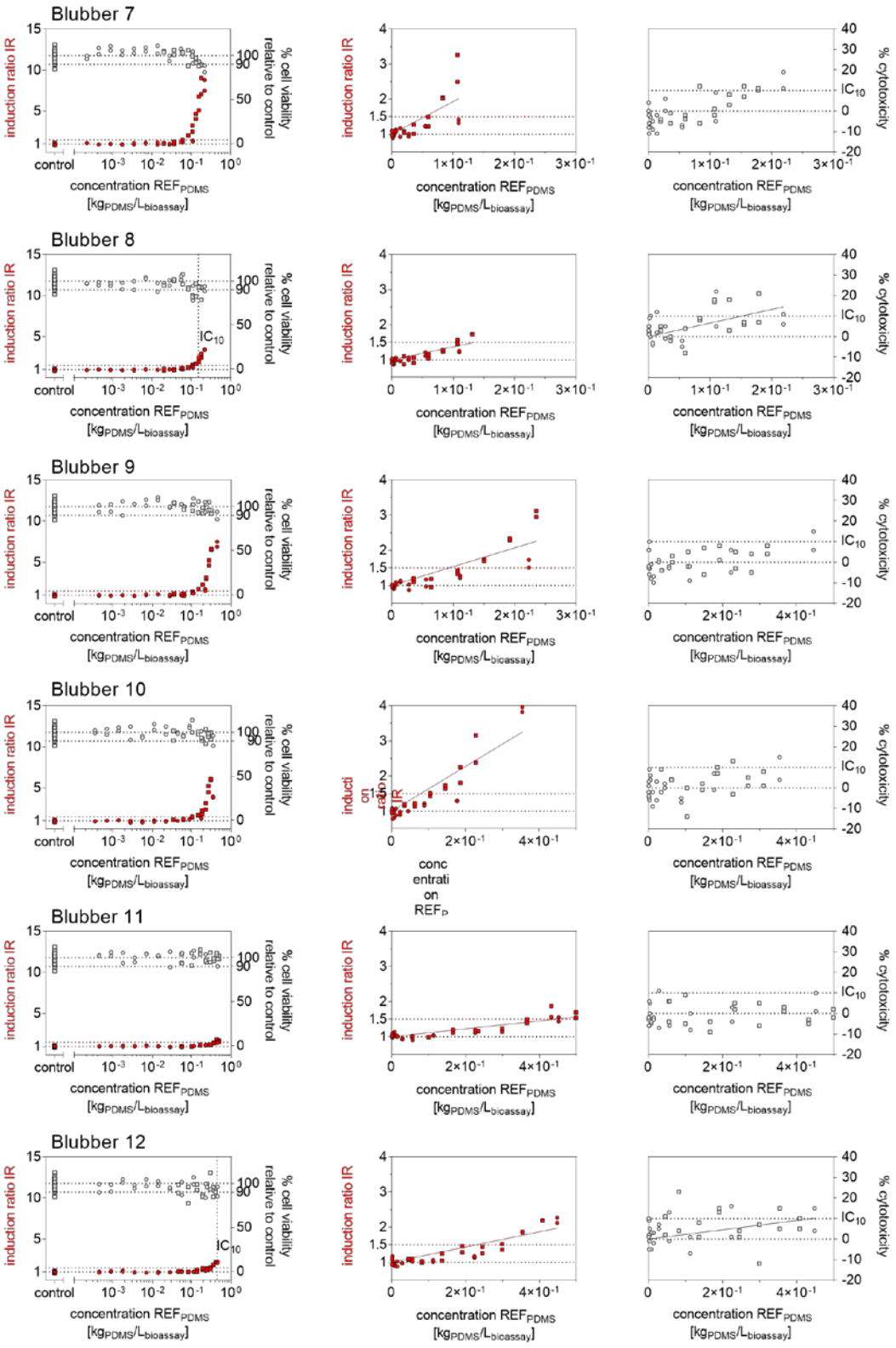
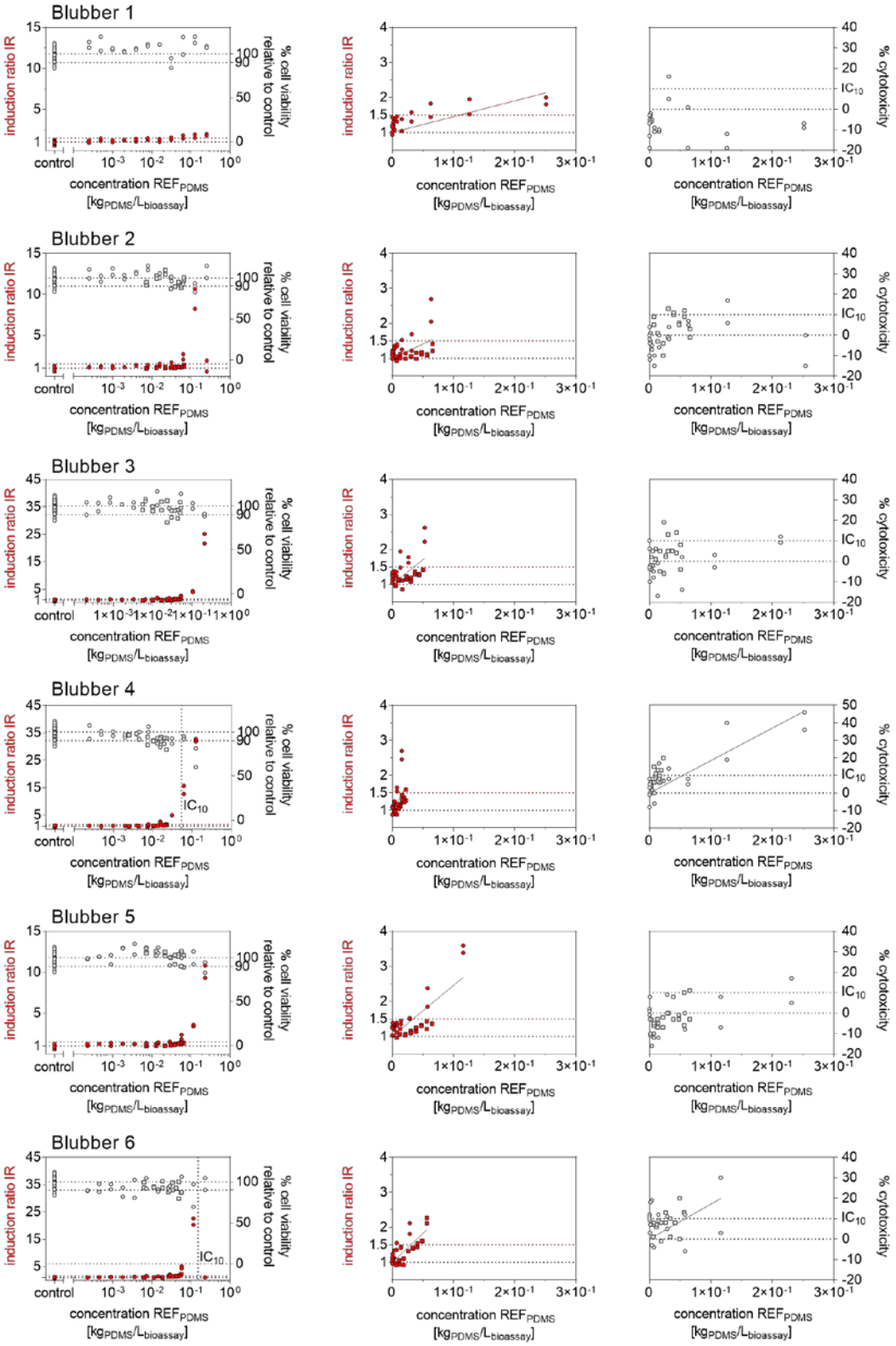


Figure SI 15. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for non-equilibrated blubber samples 1-12 (0 h). All concentrations are nominal concentrations. Blubber 1-8 from dugong, blubber 9 from ringed seal, blubber 10 from grey seal and blubber 11 and 12 from harbour porpoise.


Blubber 1
Blubber 2
Blubber 3
Blubber 4
Blubber 5
Blubber 6
induction ratio IR
% cell viability relative to control
% cytotoxicity
IC10
control
concentration REFPDMS
[kgPDMS/Lbioassay]

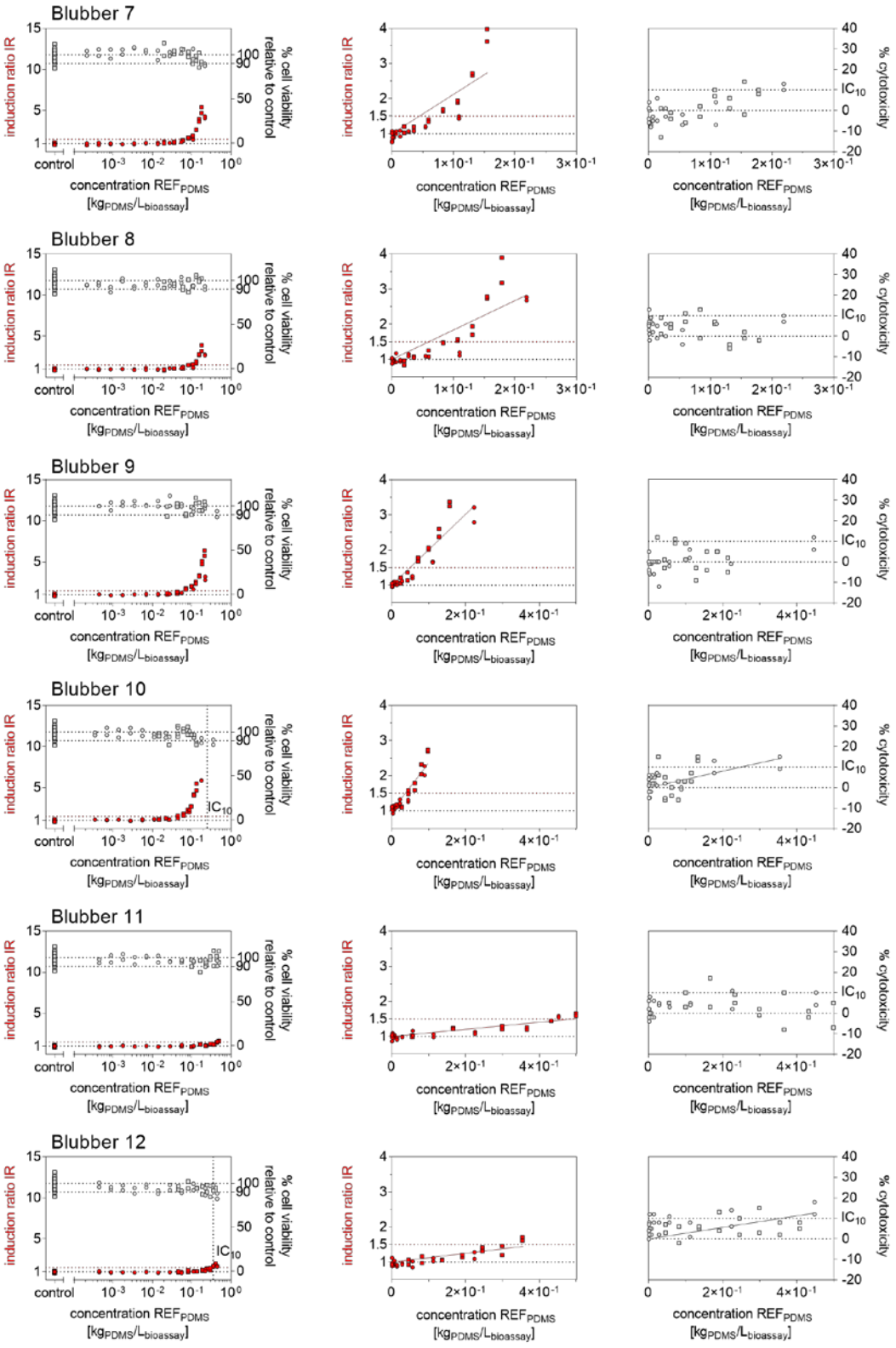


Figure SI 16. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for 24 h pre-equilibrated blubber samples 1-12 (24 h). All concentrations are nominal concentrations. Blubber 1-8 from dugong, blubber 9 from ringed seal, blubber 10 from grey seal and blubber 11 and 12 from harbour porpoise.

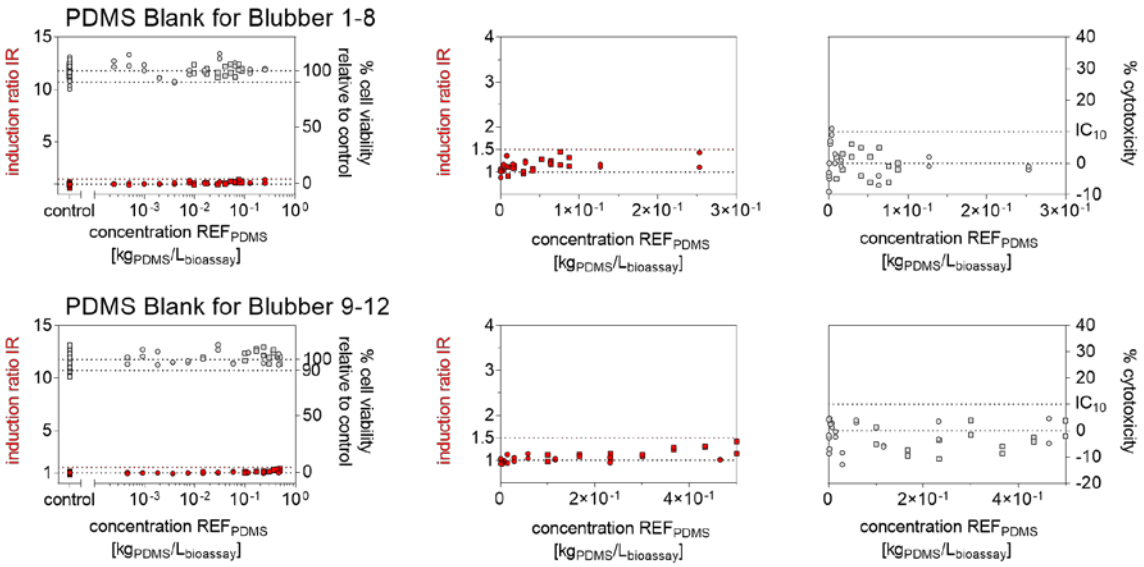


Figure SI 17. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for non-equilibrated PDMS Blanks (0 h).

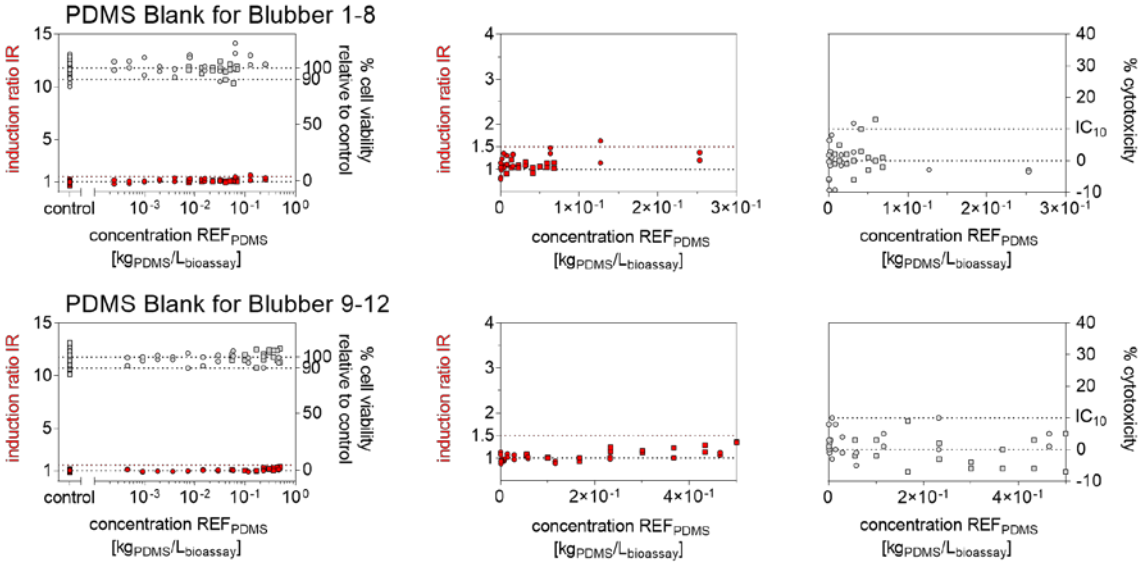


Figure SI 18. Concentration-effect curves on logarithmic and linear scales including cytotoxicity for 24 h pre-equilibrated PDMS Blanks (24 h).

Table SI 5. Mean effect concentrations $EC_{IR1.5}$ and the volume fraction V*f* of lipid at the $EC_{IR1.5}$ and inhibitory concentrations IC_{10} for cytotoxicity from blubber samples 1-12 and their associated SE are derived from the linear regression of the concentration-effect relationships shown in Figures SI 13 to SI 16. The label 'without pre-equilibration' refers to dosing directly after resuspension of the extract and medium and 'with 24h pre-equilibration' means that the blubber extract was pre-equilibrated with the medium for 24 h prior to dosing in dosing vials. For more information, see the main text. Blubber 1-8 from dugong, blubber 9 from ringed seal, blubber 10 from grey seal and blubber 11 and 12 from harbour porpoise.

Sample	without pre-equilibration			with 24 h pre-equilibration		
	lipid fraction at $EC_{IR1.5}$ [%]	$EC_{IR1.5}$ [kg_{PDMS}/ $L_{bioassay}$]	IC_{10} [kg_{PDMS}/ $L_{bioassay}$]	lipid fraction at $EC_{IR1.5}$ [%]	$EC_{IR1.5}$ [kg_{PDMS}/ $L_{bioassay}$]	IC_{10} [kg_{PDMS}/ $L_{bioassay}$]
Blubber 1	0.10	$(2.0\pm0.4)\cdot10^{-1}$	$> 2.5\cdot10^{-1}$	0.06	$(1.1\pm0.2)\cdot10^{-1}$	$> 2.5\cdot10^{-1}$
Blubber 2	0.04	$(7.8\pm0.7)\cdot10^{-2}$	$(2.2\pm0.8)\cdot10^{-1}$	0.04	$(6.1\pm0.1)\cdot10^{-2}$	$> 2.5\cdot10^{-1}$
Blubber 3	0.01	$(2.3\pm0.2)\cdot10^{-2}$	$(1.5\pm0.3)\cdot10^{-1}$	0.01	$(3.5\pm0.5)\cdot10^{-2}$	$> 2.1\cdot10^{-1}$
Blubber 4	0.01	$(8.5\pm0.6)\cdot10^{-3}$	$(5.1\pm0.3)\cdot10^{-2}$	0.03	$(1.7\pm0.3)\cdot10^{-2}$	$(5.4\pm0.5)\cdot10^{-2}$
Blubber 5	0.02	$(3.0\pm0.2)\cdot10^{-2}$	$(1.8\pm0.6)\cdot10^{-1}$	0.01	$(3.4\pm0.3)\cdot10^{-2}$	$> 2.3\cdot10^{-1}$
Blubber 6	0.02	$(4.1\pm0.9)\cdot10^{-2}$	$(5.6\pm0.6)\cdot10^{-2}$	0.02	$(3.0\pm0.3)\cdot10^{-2}$	$(5.8\pm0.1)\cdot10^{-2}$
Blubber 7	0.03	$(5.4\pm0.7)\cdot10^{-2}$	$> 2.2\cdot10^{-1}$	0.02	$(4.5\pm0.4)\cdot10^{-2}$	$> 2.2\cdot10^{-1}$
Blubber 8	0.04	$(1.4\pm0.1)\cdot10^{-1}$	$(1.5\pm0.3)\cdot10^{-1}$	0.02	$(6.0\pm0.5)\cdot10^{-2}$	$> 2.2\cdot10^{-1}$
Blubber 9	0.06	$(9.3\pm0.8)\cdot10^{-2}$	$> 4.5\cdot10^{-1}$	0.03	$(5.0\pm0.3)\cdot10^{-2}$	$> 4.5\cdot10^{-1}$
Blubber 10	0.05	$(7.9\pm0.6)\cdot10^{-2}$	$> 3.5\cdot10^{-1}$	0.02	$(3.7\pm0.2)\cdot10^{-2}$	$(2.5\pm0.5)\cdot10^{-1}$
Blubber 11	0.77	$(4.5\pm0.3)\cdot10^{-1}$	$> 4.9\cdot10^{-1}$		$> 4.9\cdot10^{-1}$	$> 4.9\cdot10^{-1}$
Blubber 12	0.30	$(2.3\pm0.1)\cdot10^{-1}$	$(4.4\pm1.2)\cdot10^{-1}$		$> IC_{10}$	$(3.6\pm0.6)\cdot10^{-1}$

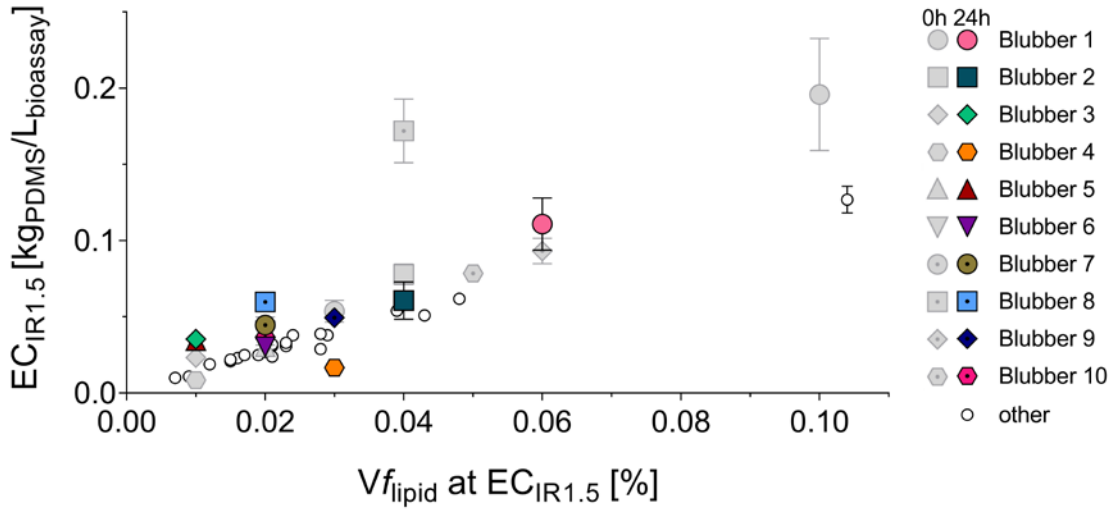


Figure SI 19. Extended representation of the relationship between $EC_{IR1.5}$ and Vf_{lipid} for blubber samples 1-10 (Figure 3, main text), showing additionally the '0 h' dosing. Twenty-four additional blubber samples from dugongs (white circles, '0 h' dosing, legend "other") are also included in the plot. They were extracted with PDMS analogously to the blubber samples 1-10 during previous experiments (unpublished). In some cases, the SE bars are smaller than the symbols.

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