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Supplemental Material

Cytotoxicity Burst? Differentiating Specific from Nonspecific Effects in Tox21 *in Vitro* Reporter Gene Assays

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References

Additional File- Excel Document

Text S1. MATLAB code 1: Processing and sorting of Tox21 concentration-response browser data

%This is the script to transform the .csv files downloaded from the Tox21 concentration-response browser into individual .csv files containing the data for each chemical for further data analysis

```
   clear all;
   M = readtable('.csv file downloaded from the browser.csv');
   outputByName = containers.Map;
   input = M;
   [rowCount, ] = size(input);
   chemNameIndex = 1;

% Now we look at each file independently
   for row = 1:rowCount
% Identify Chemical name as input
      chemName = input{row,chemNameIndex};
      name = string(chemName);
      key=char(name);
      if isKey(outputByName, key)
% Now we extract concentration, effect and CAS-Number
          outputByName(key) = [outputByName(key); input(row, 2:5)];
      else
            outputByName(key) = input(row, 2:5);
      end
   end
allKeys = keys(outputByName);
keyCount = size(allKeys,2);

% This loop writes the independent csv. files
for keyIndex = 1:keyCount
   key = allKeys{keyIndex};
   outputTable = outputByName(key);
% Now we write files named by the chemical and save them in the workspace
   writetable(outputTable, filename);
end
```

Text S2. MATLAB code 2: Calculation of IC_{10} and EC_{10} from the Tox21 concentration-response data

%This is the script to fit the cell viability and activity data yielding EC10 and IC10 values with standard errors and maximum concentrations considered in the fit. The code first excludes all values for cytotoxicity >50% effect and all >30% activity effects. Then, linear regression is fitted and with resulting slopes. The IC10 and EC10 are then 0.1/slope.

```
clear all;
pathxls='add path containing individual .csv files from Code 1';
d=uigetdir('add path containing individual .csv files from Code 1');
cd(d);
pr=dir('*.xls');
names={pr.name};
% First, we need to create empty matrices for the values we want to calculate that we can later fill with data
IC10 = [];
EC10 = [];
SE_IC10 = [];
SE_EC10 = [];
CAS = [];
Max_conc_EC10 = [];
Max_conc_IC10 = [];

for NameIndex=1:length(names)
   NUMERIC=readtable(names{NameIndex});
   x_viab=[ ];
   y_viab=[ ];
   x_ratio=[ ];
   y_ratio=[ ];

   for Index=1:height(NUMERIC)
      experiment=string(NUMERIC{Index,1});
      if startsWith(experiment, 'ratio')
         if NUMERIC{Index, 3} < 30
   x_ratio = [x_ratio;NUMERIC{Index, 2}];
   y_ratio = [y_ratio;NUMERIC{Index, 3}];
         end
elseif startsWith(experiment, 'via')
   if NUMERIC{Index, 3} < 50
   x_viab = [x_viab;NUMERIC{Index, 2}];
```

```
    y_viab = [y_viab;NUMERIC{Index, 3}];
    end
        else
            disp('Experiment not recognized')
        end
    end
    x_viabdelog = 10.^x_viab*1000000;
    x_ratiodelog = 10.^x_ratio*1000000;
    y_viabdelog = y_viab;
    y_ratiodelog = y_ratio;
    b_viab = x_viabdelog\y_viabdelog;
    b_ratio = x_ratiodelog\y_ratiodelog;
    y_viabfit = x_viabdelog*b_viab;
    y_ratiofit = x_ratiodelog*b_ratio;
    r_viab = y_viabdelog-y_viabfit;
    r_ratio = y_ratiodelog-y_ratiofit;
    n_viab = length(x_viabdelog);
    n_ratio = length(x_ratiodelog);
    SSE_viab = sum(r_viab.^2);
    SSE_ratio = sum(r_ratio.^2);
    MSE_viab=SSE_viab/(n_viab-1);
    MSE_ratio=SSE_ratio/(n_ratio-1);
    SE_viab = sqrt(MSE_viab/sum(x_viabdelog.^2));
    SE_ratio = sqrt(MSE_ratio/sum(x_ratiodelog.^2));
    SE_IC10 = [SE_IC10; 10/b_viab^2*SE_viab];
    SE_EC10 = [SE_EC10; 10/b_ratio^2*SE_ratio];
    IC10 = [IC10; 10/b_viab];
    EC10 = [EC10; 10/b_ratio];
    CAS = [CAS; NUMERIC{1,4}]
    Max_conc_EC10 = [Max_conc_EC10; max(x_ratiodelog)];
    Max_conc_IC10 = [Max_conc_IC10; max(x_viabdelog)];
end

Output = table(transpose(names), CAS, IC10, SE_IC10, EC10, SE_EC10, Max_conc_EC10,
Max_conc_IC10);
writetable(Output, 'define output sheet name here.xls');
```

Table S1. Additional information on test chemicals.

Chemical	CAS Number	DSSTox	$f_{neutral}$	log $K_{ip/w}$ or log $D_{lip/w}$ (pH 7.4) [L/L]	Source	Purity
2-Phenylphenol	90-43-7	DTXSID2021151	1.00	3.46[a]	Sigma Aldrich	99.6%
3-Nitroaniline	99-09-2	DTXSID6025725	1.00	2.17[a]	Sigma-Aldrich	98.0%
4-Chloro-3-methylphenol	59-50-7	DTXSID4021717	1.00	3.34[a]	Sigma-Aldrich	≥ 98%
4-Pentylphenol[b]	14938-35-3	DTXSID9044164	1.00	4.31[a]	Sigma-Aldrich	≥ 98%
2-Allylphenol	1745-81-9	DTXSID3022164	1.00	3.06[a]	Sigma-Aldrich	98.0%
2-Butoxyethanol	111-76-2	DTXSID1024097	1.00	0.60[a]	Sigma-Aldrich	≥ 99.5%
2,4,5-Trichloroaniline[b]	636-30-6	DTXSID5044152	1.00	4.16[a]	Sigma-Aldrich	99.1%
Bisphenol A	80-05-7	DTXSID1048839	0.99	3.50[c]	Sigma-Aldrich	>99%
Quinoxyfen	124495-18-7	DTXSID1042364	1.00	5.32[d]	Sigma-Aldrich	Pestanal, analytical standard
Fluoranthene	206-44-0	DTXSID8020628	1.00	5.41[e]	Sigma-Aldrich	98.0%
Genistein	446-72-0	DTXSID3047849	0.39	3.32[f]	Roth	>98%
Coumarin	91-64-5	DTXSID8041797	1.00	2.09[d]	Sigma-Aldrich	>99%
8-Gingerol[b]	23513-08-8	DTXSID20178078	1.00	4.70[d]	Sigma-Aldrich	>98%
Zingerone[b]	122-48-5	DTXSID8047420	1.00	1.97[d]	Sigma-Aldrich	>96%

[a](Vaes et al. 1997).

[b]Not included in Tox21.

[c](Kwon et al. 2006).

[d](Henneberger et al. 2020).

[e](van der Heijden and Jonker 2009).

[f](Henneberger et al. 2019).

Table S2. Analysis of the specific effects of the baseline toxicants in AREc32.

AREc32	IC_{10} (M)	$EC_{IR1.5}$ (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$	Specificity ratio $SR_{baseline}$
2-Phenylphenol	$9.9\pm0.4 \cdot 10^{-5}$	$9.5\pm0.4 \cdot 10^{-5}$	2.0	1.0	2.1
3-Nitroaniline	$1.7\pm0.2 \cdot 10^{-3}$	$3.8\pm0.2 \cdot 10^{-3}$	0.6	4.3	2.8
4-Chloro-3-methylphenol	$1.3\pm0.1 \cdot 10^{-4}$	$1.4\pm0.1 \cdot 10^{-4}$	1.9	0.9	1.7
4-Pentylphenol	$7.9\pm0.8 \cdot 10^{-5}$	$5.0\pm0.2 \cdot 10^{-5}$	0.8	1.6	1.3
2-Allylphenol	$2.8\pm0.4 \cdot 10^{-4}$	$1.7\pm0.6 \cdot 10^{-4}$	1.2	1.6	1.9
2-Butoxyethanol	$9.2\pm0.4 \cdot 10^{-3}$	$3.0\pm0.6 \cdot 10^{-2}$	0.9	0.3	0.3
2,4,5-Trichloroaniline	$2.1\pm0.2 \cdot 10^{-4}$	$1.3\pm0.4 \cdot 10^{-4}$	0.4	1.6	0.6

Note: we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the $EC_{IR1.5}$ values to demonstrate the cytotoxicity burst.

Abbreviations: $EC_{IR1.5}$, effect concentration causing an induction ratio IR of 1.5 (50% over control); IC_{10}, 10% inhibitory concentration (cytotoxicity). $EC_{IR1.5}$ and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve.

Table S3. Analysis of the specific effects of the baseline toxicants in ARE-BLA.

ARE-BLA	IC_{10} (M)	$EC_{IR1.5}$ (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$	Specificity ratio $SR_{baseline}$
2-Phenylphenol	$1.3\pm0.1 \cdot 10^{-4}$	$1.3\pm0.7 \cdot 10^{-4}$	2.2	1.0	2.2
3-Nitroaniline	$2.1\pm0.2 \cdot 10^{-3}$	$4.3\pm0.2 \cdot 10^{-4}$	0.5	5.0	2.5
4-Chloro-3-methylphenol	$2.3\pm0.1 \cdot 10^{-4}$	no effect	1.0	-	-
4-Pentylphenol	$6.0\pm0.4 \cdot 10^{-5}$	no effect	1.1	-	-
2-Allylphenol	$1.2\pm0.5 \cdot 10^{-4}$	$5.4\pm0.3 \cdot 10^{-4}$	0.3	2.2	0.6
2-Butoxyethanol	$2.4\pm0.1 \cdot 10^{-2}$	$3.6\pm0.3 \cdot 10^{-4}$	0.3	0.7	0.2
2,4,5-Trichloroaniline	$1.6\pm0.1 \cdot 10^{-4}$	$3.3\pm0.1 \cdot 10^{-4}$	0.5	0.5	0.2

Note: that we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the $EC_{IR1.5}$ values to demonstrate the cytotoxicity burst.

Abbreviations: $EC_{IR1.5}$, effect concentration causing an induction ratio IR of 1.5 (50% over control); IC_{10}, 10% inhibitory concentration (cytotoxicity). $EC_{IR1.5}$ and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed induction ratio IR 1.5.

Table S4. Analysis of the specific effects of the baseline toxicants in AhR-CALUX.

AhR-CALUX	IC_{10} (M)	EC_{10} (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$	Specificity ratio $SR_{baseline}$
2-Phenylphenol	$1.3\pm0.9\cdot10^{-4}$	$1.3\pm0.4\cdot10^{-4}$	1.2	1.0	1.2
3-Nitroaniline	$4.1\pm0.3\cdot10^{-4}$	no effect	3.3	-	-
4-Chloro-3-methylphenol	$1.6\pm0.4\cdot10^{-4}$	$1.6\pm0.1\cdot10^{-4}$	1.2	1.0	1.2
4-Pentylphenol	$4.9\pm0.3\cdot10^{-5}$	$1.2\pm0.1\cdot10^{-4}$	0.8	0.4	0.3
2-Allylphenol	$4.7\pm0.6\cdot10^{-4}$	$5.1\pm0.1\cdot10^{-4}$	0.7	0.9	0.6
2-Butoxyethanol	$3.8\pm0.9\cdot10^{-2}$	too variable	0.5	-	-
2,4,5-Trichloroaniline	$7.8\pm0.7\cdot10^{-5}$	$9.3\pm0.2\cdot10^{-6}$	1.1	8.1	8.7

Note: that we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the $EC_{IR1.5}$ values to demonstrate the cytotoxicity burst.

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10%; too variable: concentration-response curves of different independent repeats were inconclusive because the differed too much, no EC_{10} could be derived.

Table S5. Analysis of the specific effects of the baseline toxicants in PPARγ-BLA.

PPARγ-BLA	IC_{10} (M)	EC_{10} (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$	Specificity ratio $SR_{baseline}$
2-Phenylphenol	$5.4\pm0.3\cdot10^{-5}$	no effect	2.2	-	-
3-Nitroaniline	-	no effect	-	-	-
4-Chloro-3-methylphenol	$3.3\pm0.2\cdot10^{-5}$	$1.6\pm0.1\cdot10^{-5}$	5.8	0.2	1.2
4-Pentylphenol	$1.5\pm0.5\cdot10^{-4}$	$1.2\pm0.1\cdot10^{-4}$	2.5	0.1	0.3
2-Allylphenol	$6.4\pm0.3\cdot10^{-4}$	$5.1\pm0.1\cdot10^{-4}$	0.5	1.3	0.6
2-Butoxyethanol	$8.8\pm0.4\cdot10^{-3}$	no effect	2.2	-	-
2,4,5-Trichloroaniline	$2.6\pm0.7\cdot10^{-4}$	$9.3\pm0.2\cdot10^{-6}$	0.3	28.2	8.7

Note: that we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the EC_{10} values to demonstrate the cytotoxicity burst.

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10%.

Table S6. Analysis of the specific effects of the baseline toxicants in AR-BLA.

AR-BLA	IC_{10} (M)	EC_{10} (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$
2-Phenylphenol	$6.6\pm0.3 \cdot 10^{-5}$	too variable	1.3	-
3-Nitroaniline	-	no effect	-	-
4-Chloro-3-methylphenol	$2.5\pm0.1 \cdot 10^{-5}$	no effect	7.5	-
4-Pentylphenol	$1.4\pm0.1 \cdot 10^{-4}$	no effect	2.7	-
2-Allylphenol	$2.2\pm0.1 \cdot 10^{-4}$	no effect	0.8	-
2-Butoxyethanol	$1.4\pm0.1 \cdot 10^{-2}$	no effect	1.4	-
2,4,5-Trichloroaniline	$6.9\pm0.5 \cdot 10^{-5}$	no effect	1.2	-

Note: that we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the EC_{10} values to demonstrate the cytotoxicity burst.

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10% effect; too variable: concentration-response curves of different independent repeats were inconclusive because the differed too much, no EC_{10} could be derived.

Table S7. Analysis of the specific effects of the baseline toxicants in ERα-BLA.

ERα-BLA	IC_{10} (M)	$EC_{IR1.5}$ (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$	Specificity ratio $SR_{baseline}$
2-Phenylphenol	$5.6\pm0.3 \cdot 10^{-5}$	no effect	1.2	-	-
3-Nitroaniline	$2.5\pm0.4 \cdot 10^{-4}$	no effect	5.6	-	-
4-Chloro-3-methylphenol	$2.6\pm0.1 \cdot 10^{-5}$	$2.4\pm0.3 \cdot 10^{-5}$	7.3	1.1	8.1
4-Pentylphenol	$1.3\pm0.1 \cdot 10^{-5}$	$3.2\pm1.1 \cdot 10^{-5}$	3.0	0.4	1.2
2-Allylphenol	$4.1\pm0.3 \cdot 10^{-4}$	too variable	1.4	-	-
2-Butoxyethanol	$1.9\pm0.4 \cdot 10^{-2}$	too variable	1.0	-	-
2,4,5-Trichloroaniline	$6.3\pm0.3 \cdot 10^{-5}$	no effect	1.3	-	-

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10% effect; too variable: concentration-response curves of different independent repeats were inconclusive because the differed too much, no EC_{10} could be derived.

Table S8. Analysis of the specific effects of the baseline toxicants in PR-BLA.

PR-BLA	IC_{10} (M)	$EC_{IR1.5}$ (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$	Specificity ratio $SR_{baseline}$
2-Phenylphenol	$5.7\pm0.2\cdot10^{-5}$	no effect	2.0	-	-
3-Nitroaniline	$(7.0\pm0.4\cdot10^{-5})$	no effect	-	-	-
4-Chloro-3-methylphenol	$2.7\pm0.1\cdot10^{-5}$	no effect	5.2	-	-
4-Pentylphenol	$3.1\pm0.2\cdot10^{-5}$	no effect	0.9	-	-
2-Allylphenol	$5.2\pm0.3\cdot10^{-4}$	no effect	0.4	-	-
2-Butoxyethanol	$1.4\pm0.1\cdot10^{-2}$	$1.3\pm0.1\cdot10^{-2}$	0.8	1.1	0.9
2,4,5-Trichloroaniline	$9.5\pm0.5\cdot10^{-5}$	no effect	0.4	-	

Note: that we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the EC_{10} values to demonstrate the cytotoxicity burst.

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10% effect.

Table S9. Analysis of the specific effects of the baseline toxicants in GR-BLA.

GR-BLA	IC_{10} (M)	$EC_{IR1.5}$ (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$
2-Phenylphenol	$4.4\pm0.1\cdot10^{-5}$	no effect	1.6	-
3-Nitroaniline	$(7.3\pm0.3\cdot10^{-5})$	no effect		-
4-Chloro-3-methylphenol	$2.4\pm0.1\cdot10^{-5}$	no effect	5.9	-
4-Pentylphenol	$2.3\pm0.2\cdot10^{-4}$	no effect	1.2	-
2-Allylphenol	$2.3\pm0.1\cdot10^{-4}$	no effect	1.0	-
2-Butoxyethanol	$1.0\pm0.1\cdot10^{-2}$	no effect	1.1	-
2,4,5-Trichloroaniline	$4.7\pm0.2\cdot10^{-5}$	no effect	0.8	-

Note: that we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the EC_{10} values to demonstrate the cytotoxicity burst.

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10% effect.

Table S10. Analysis of the specific effects of the environmental chemicals in AREc32.

AREc32	IC_{10} (M)	$EC_{IR1.5}$ (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$	Specificity ratio $SR_{baseline}$
Bisphenol A	$7.2\pm05\cdot10^{-5}$	$4.9\pm0.2\cdot10^{-5}$	2.8	1.5	4.0
Quinoxyfen	$2.1\pm0.2\cdot10^{-5}$	$1.5\pm0.2\cdot10^{-5}$	1.9	1.5	1.3
Fluoranthene	$8.5\pm1.1\cdot10^{-5}$	$5.6\pm0.5\cdot10^{-5}$	0.2	1.5	0.3
Genistein	$2.1\pm0.3\cdot10^{-4}$	$5.3\pm0.3\cdot10^{-6}$	1.1	38	47
Coumarin	$2.3\pm0.5\cdot10^{-3}$	$5.6\pm0.2\cdot10^{-4}$	0.7	4.2	2.1
8-Gingerol	$6.1\pm0.4\cdot10^{-5}$	no effect	0.3	-	-
Zingerone	$1.5\pm0.8\cdot10^{-3}$	$4.8\pm0.3\cdot10^{-4}$	0.5	3.0	2.9

Note: we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the $EC_{IR1.5}$ values to demonstrate the cytotoxicity burst.

Abbreviations: $EC_{IR1.5}$, effect concentration causing an induction ratio IR of 1.5 (50% over control); IC_{10}, 10% inhibitory concentration (cytotoxicity). $EC_{IR1.5}$ and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed an induction ratio IR of 1.5.

Table S11. Analysis of the specific effects of the environmental chemicals in ARE-BLA.

ARE-BLA	IC_{10} (M)	$EC_{IR1.5}$ (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$	Specificity ratio $SR_{baseline}$
Bisphenol A	$1.3\pm0.1 \cdot 10^{-4}$	$1.2\pm0.1 \cdot 10^{-4}$	2.9	1.1	2.3
Quinoxyfen	$1.3\pm0.1 \cdot 10^{-5}$	$3.1\pm0.1 \cdot 10^{-5}$	0.3	4.1	0.5
Fluoranthene	no cytotoxicity	$1.5\pm0.1 \cdot 10^{-4}$	-	-	0.1
Genistein	$3.2\pm0.4 \cdot 10^{-4}$	$4.0\pm0.2 \cdot 10^{-4}$	1.0	8.3	9.0
Coumarin	$3.6\pm0.2 \cdot 10^{-3}$	$1.6\pm0.1 \cdot 10^{-3}$	1.0	2.3	1.6
8-Gingerol	$5.5\pm0.3 \cdot 10^{-5}$	no effect	0.3	-	-
Zingerone	$2.8\pm0.2 \cdot 10^{-3}$	no effect	0.5	-	-

Note: we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the $EC_{IR1.5}$ values to demonstrate the cytotoxicity burst.

Abbreviations: $EC_{IR1.5}$, effect concentration causing an induction ratio IR of 1.5 (50% over control); IC_{10}, 10% inhibitory concentration (cytotoxicity). $EC_{IR1.5}$ and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed an induction ratio IR of 1.5.

Table S12. Analysis of the specific effects of the environmental chemicals in AhR-CALUX.

AhR-CALUX	IC_{10} (M)	EC_{10}(M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$	Specificity ratio $SR_{baseline}$
Bisphenol A	$1.2\pm0.1 \cdot 10^{-4}$	$1.2\pm0.4 \cdot 10^{-4}$	1.2	1.0	1.2
Quinoxyfen	$1.8\pm0.1 \cdot 10^{-5}$	no effect	1.1	-	-
Fluoranthene	$1.3\pm0.1 \cdot 10^{-4}$	$1.7\pm0.1 \cdot 10^{-5}$	0.049	7.5	0.3
Genistein	$3.0\pm0.3 \cdot 10^{-5}$	$1.9\pm0.1 \cdot 10^{-5}$	6.8	1.6	10.6
Coumarin	$8.1\pm0.3 \cdot 10^{-4}$	no effect	2.9	-	-
8-Gingerol	$5.8\pm0.6 \cdot 10^{-5}$	no effect	0.1	-	-
Zingerone	$5.8\pm031 \cdot 10^{-4}$	$3.5\pm0.5 \cdot 10^{-4}$	1.6	1.7	5.5

Note: we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the EC_{10} values to demonstrate the cytotoxicity burst.

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10% effect.

Table S13. Analysis of the specific effects of the environmental chemicals in PPARγ-BLA.

PPARγ-BLA	IC_{10} (M)	EC_{10} (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$	Specificity ratio $SR_{baseline}$
Bisphenol A	$2.6\pm0.2 \cdot 10^{-5}$	no effect	4.2	-	-
Quinoxyfen	$1.8\pm0.2 \cdot 10^{-5}$	$7.1\pm0.1 \cdot 10^{-7}$	1.0	25.4	10.8
Fluoranthene	no cytotoxicity	$1.3\pm0.7 \cdot 10^{-5}$	-	-	0.5
Genistein	$2.3\pm0.1 \cdot 10^{-5}$	no effect	6.3	-	-
Coumarin	$2.3\pm0.2 \cdot 10^{-3}$	$8.6\pm0.1 \cdot 10^{-4}$	0.6	1.5	1.0
8-Gingerol	$4.6\pm0.3 \cdot 10^{-5}$	no effect	0.2	-	-
Zingerone	$8.7\pm0.3 \cdot 10^{-4}$	no effect	0.6	-	-

Note: we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the EC_{10} values to demonstrate the cytotoxicity burst.

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±)

standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10% effect.

Table S14. Analysis of the specific effects of the environmental chemicals in AR-BLA.

AR-BLA	IC_{10} (M)	$EC_{IR1.5}$ (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$
Bisphenol A	$2.2\pm0.1 \cdot 10^{-5}$	no effect	3.5	-
Quinoxyfen	$2.12\pm0.4 \cdot 10^{-5}$	no effect	0.4	-
Fluoranthene	no cytotoxicity	no effect	-	-
Genistein	$2.5\pm0.1 \cdot 10^{-5}$	no effect	4.3	-
Coumarin	$2.9\pm0.6 \cdot 10^{-3}$	no effect	0.5	-
8-Gingerol	$1.3\pm0.8 \cdot 10^{-5}$	no effect	0.3	-
Zingerone	$1.3\pm0.9 \cdot 10^{-3}$	no effect	0.4	-

Note: we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the EC_{10} values to demonstrate the cytotoxicity burst.

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10% effect.

Table S15. Analysis of the specific effects of the environmental chemicals in ERα-BLA.

ERα-BLA	IC_{10} (M)	$EC_{IR1.5}$ (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$	Specificity ratio $SR_{baseline}$
Bisphenol A	$2.2\pm0.8 \cdot 10^{-5}$	$2.2\pm0.1 \cdot 10^{-7}$	2.9	98	282
Quinoxyfen	$2.2\pm0.2 \cdot 10^{-5}$	$4.8\pm0.4 \cdot 10^{-6}$	0.3	4.7	0.5
Fluoranthene	$3.0\pm0.2 \cdot 10^{-5}$	$6.4\pm0.1 \cdot 10^{-6}$	0.1	4.7	0.3
Genistein	$2.3\pm0.1 \cdot 10^{-4}$	$2.0\pm0.1 \cdot 10^{-7}$	3.7	118	441
Coumarin	$1.2\pm0.1 \cdot 10^{-3}$	no effect	0.9	-	-
8-Gingerol	$1.1\pm0.1 \cdot 10^{-5}$	no effect	0.3	-	-
Zingerone	$8.4\pm0.3 \cdot 10^{-3}$	$1.3\pm0.1 \cdot 10^{-4}$	0.5	6.5	7.0

Note: we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the EC_{10} values to demonstrate the cytotoxicity burst.

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10% effect.

Table S16. Analysis of the specific effects of the environmental chemicals in PR-BLA.

PR-BLA	IC_{10} (M)	$EC_{IR1.5}$ (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$
Bisphenol A	$2.4\pm0.1 \cdot 10^{-5}$	no effect	6.1	**-**
Quinoxyfen	$1.1\pm0.1 \cdot 10^{-5}$	no effect	1.9	-
Fluoranthene	no cytotoxicity	no effect		**-**
Genistein	$1.3\pm0.1 \cdot 10^{-5}$	no effect	14.9	-
Coumarin	$8.3\pm0.3 \cdot 10^{-4}$	no effect	2.3	-
8-Gingerol	$7.9\pm0.1 \cdot 10^{-6}$	no effect	1.2	-
Zingerone	$8.7\pm0.5 \cdot 10^{-4}$	no effect	0.9	**-**

Note: that we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the EC_{10} values to demonstrate the cytotoxicity burst.

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10% effect.

Table S17. Analysis of the specific effects of the environmental chemicals in GR-BLA.

GR-BLA	IC_{10} (M)	$EC_{IR1.5}$ (M)	Toxic ratio TR	Specificity ratio $SR_{cytotoxicity}$
Bisphenol A	$1.6\pm0.1 \cdot 10^{-5}$	no effect	5.5	**-**
Quinoxyfen	$1.0\pm0.1 \cdot 10^{-5}$	no effect	1.2	-
Fluoranthene	$3.0\pm0.3 \cdot 10^{-5}$	no effect	0.1	**-**
Genistein	$1.2\pm0.1 \cdot 10^{-5}$	no effect	9.8	-
Coumarin	$6.6\pm0.2 \cdot 10^{-4}$	no effect	1.9	-
8-Gingerol	$7.9\pm0.2 \cdot 10^{-6}$	no effect	0.7	-
Zingerone	$5.9\pm0.2 \cdot 10^{-4}$	no effect	0.8	**-**

Note: that we did not remove all concentrations above IC_{10} as it would be normally done in such an evaluation (Escher et al. 2018) but kept more data in the linear range to extrapolate the EC_{10} values to demonstrate the cytotoxicity burst.

Abbreviations: EC_{10}, effect concentration causing 10% of maximum effect; IC_{10}, 10% inhibitory concentration (cytotoxicity). EC_{10} and IC_{10} are reported as molar concentrations (M) plus/minus (±) standard error of mean (SEM), from error propagation of the SEM of the slope of the concentration-response curve. No effect: concentration-response curve did not exceed 10% effect.

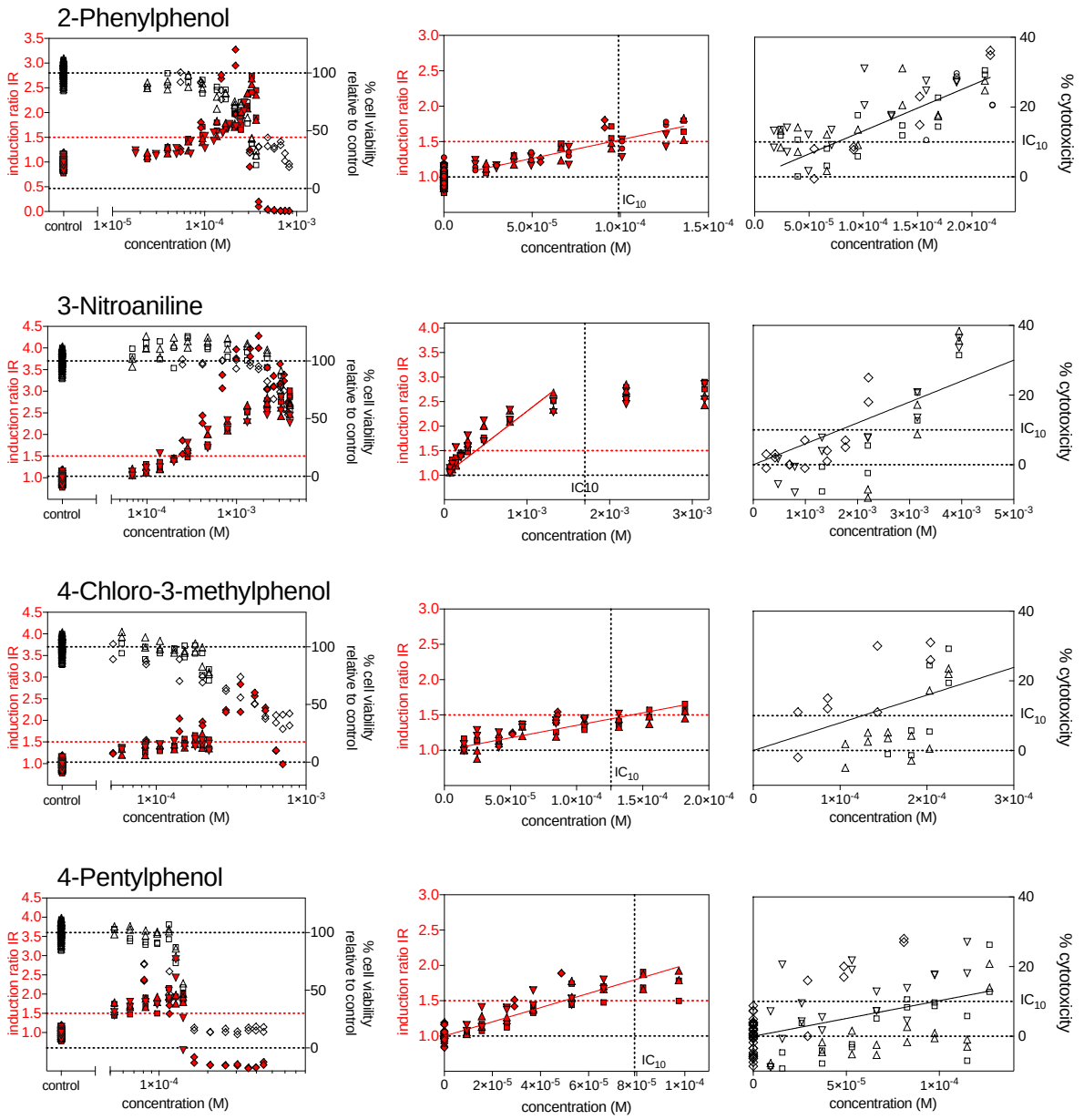
Table S18. Analysis given in Table 2 split into the two subsets of neutral ($f_{neutral}$ > 98%) and (partially) charged ($f_{neutral}$ < 98%) chemicals.

Reporter gene assay	n(TR)	n(TR > 10)		n(neutral)	n(TR > 10)		n(charged)	n(TR > 10)	
ARE-BLA	694	384	(55.3%)	340	157	(46.2%)	354	227	(64.1%)
AhR-CALUX	930	524	(56.3%)	365	165	(45.2%)	565	359	(63.5%)
PPARγ-BLA	723	326	(45.1%)	334	129	(38.6%)	389	197	(50.6%)
AR-BLA	1172	485	(41.4%)	643	226	(35.1%)	529	259	(49.0%)
ERα-BLA	610	193	(31.6%)	279	78	(28.0%)	331	115	(34.7%)
GR-BLA	599	204	(34.1%)	336	103	(30.7%)	263	101	3(8.4%)

Number of chemicals n for which a TR could be derived n(TR) and thereof number of neutral (n(neutral)) and charged (n(charged)) chemicals and number with TR > 10 (n(TR >10)).

Figure S1. Concentration-cytotoxicity and concentration-effect relationships of the confirmed baseline toxicants in AREc32.

On the left plot are all data plotted on a logarithmic concentration scale with the induction ratio of AREc32 represented in red filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the $EC_{IR1.5}$ is derived (IR 1.5 effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



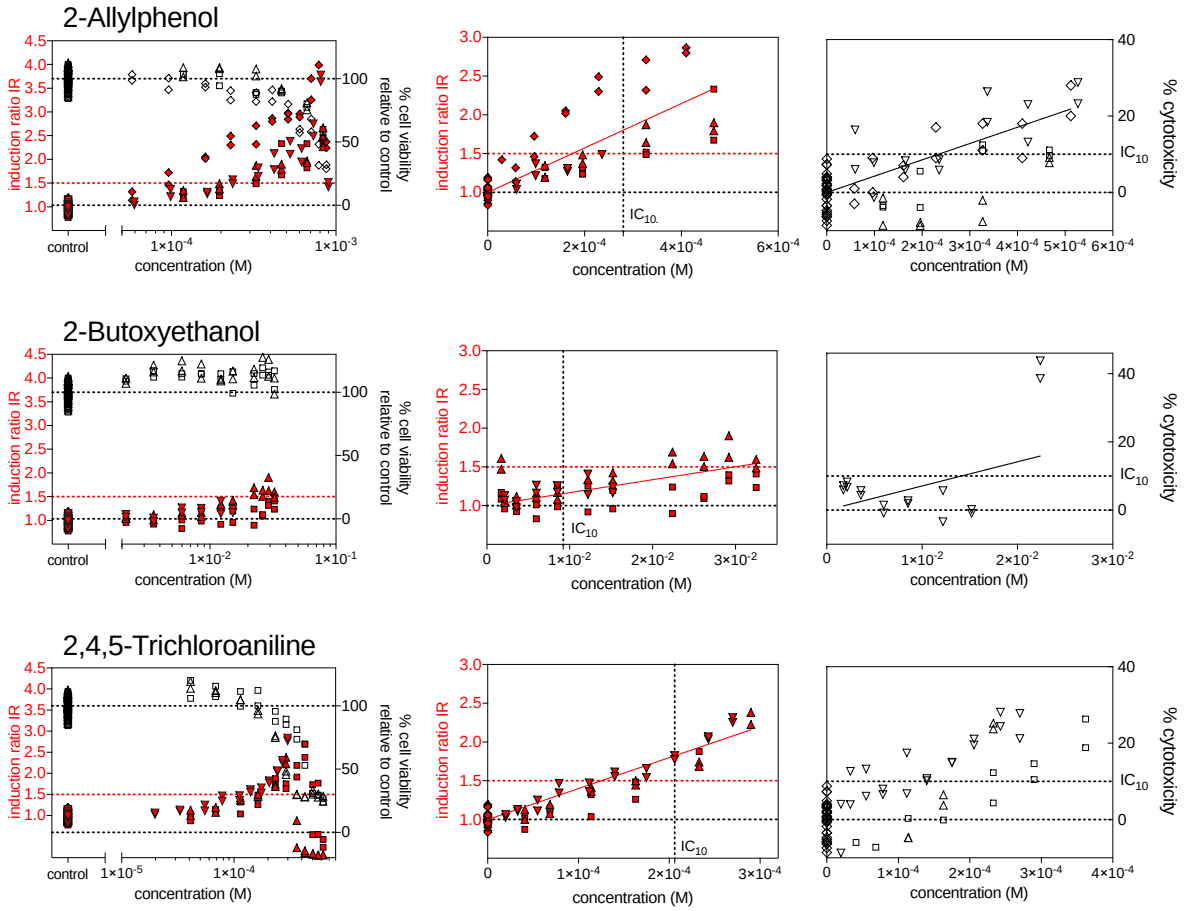
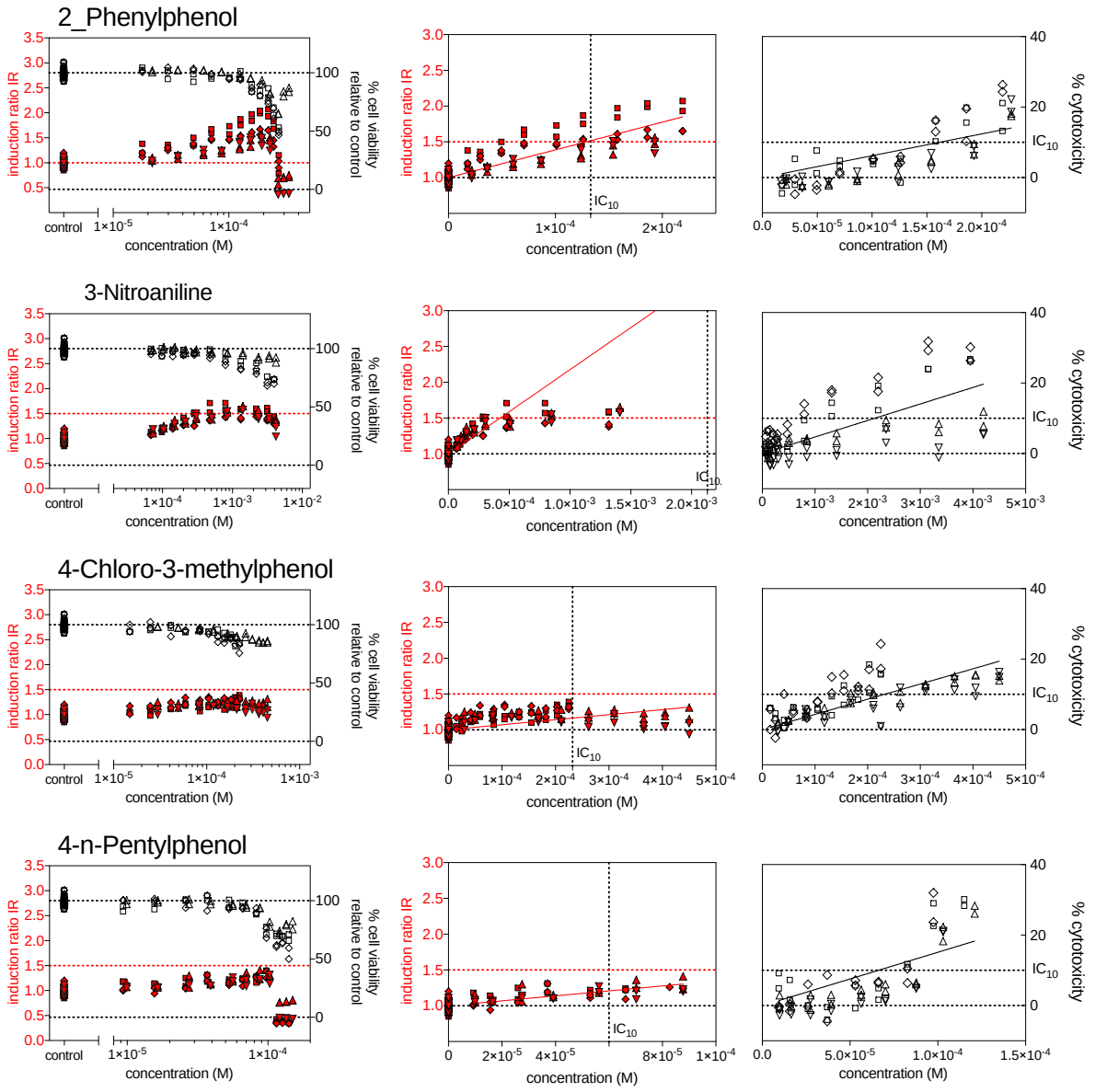

2-Allylphenol
induction ratio IR
% cell viability relative to control
control
concentration (M)
IC10
% cytotoxicity
2-Butoxyethanol
induction ratio IR
% cell viability relative to control
control
concentration (M)
IC10
% cytotoxicity
2,4,5-Trichloroaniline
induction ratio IR
% cell viability relative to control
control
concentration (M)
IC10
% cytotoxicity

Figure S2. Concentration-cytotoxicity and concentration-effect relationships of the confirmed baseline toxicants in ARE-BLA.

On the left plot are all data plotted on a logarithmic concentration scale with the induction ratio of ARE-BLA represented in red filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the $EC_{IR1.5}$ is derived (IR 1.5 effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



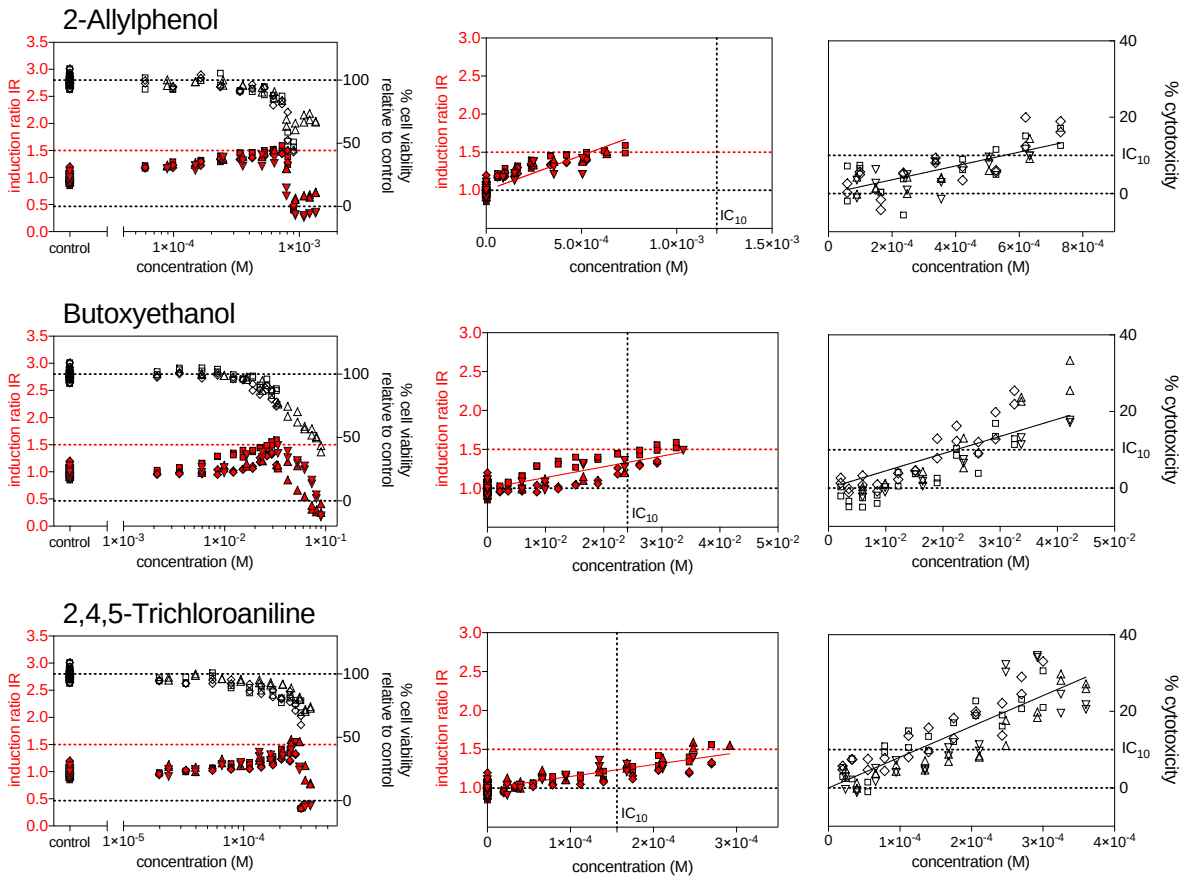
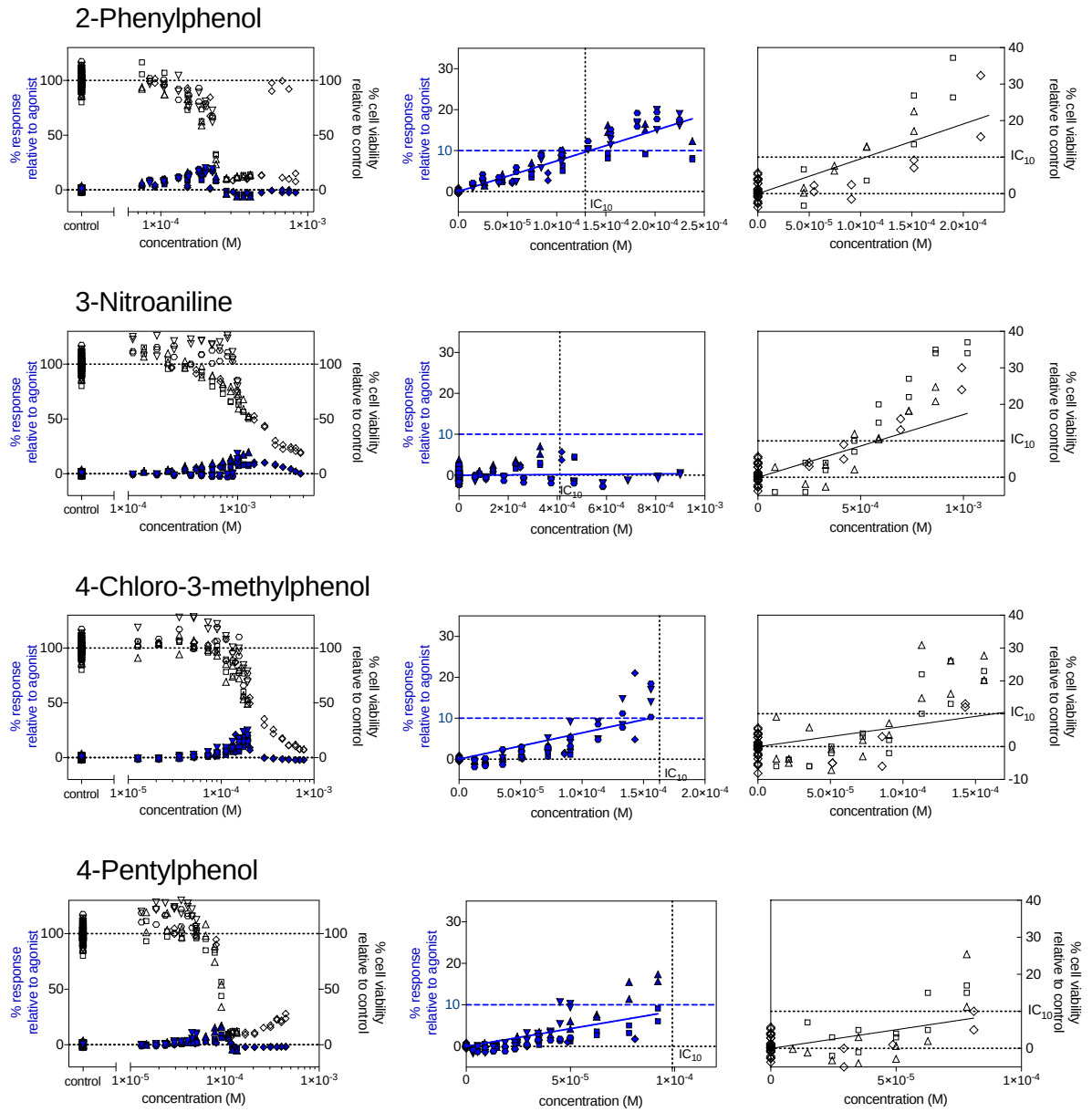
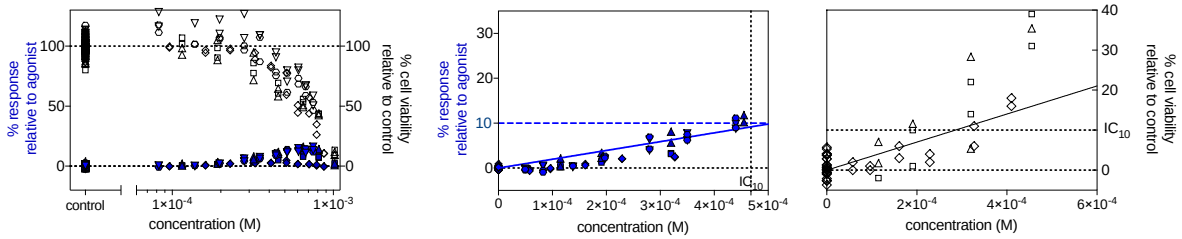

2-Allylphenol
induction ratio IR
% cell viability relative to control
control
concentration (M)
IC10
% cytotoxicity
Butoxyethanol
induction ratio IR
% cell viability relative to control
control
concentration (M)
IC10
% cytotoxicity
2,4,5-Trichloroaniline
induction ratio IR
% cell viability relative to control
control
concentration (M)
IC10
% cytotoxicity

Figure S3. Concentration-cytotoxicity and concentration-effect relationships of the confirmed baseline toxicants in AhR-CALUX.

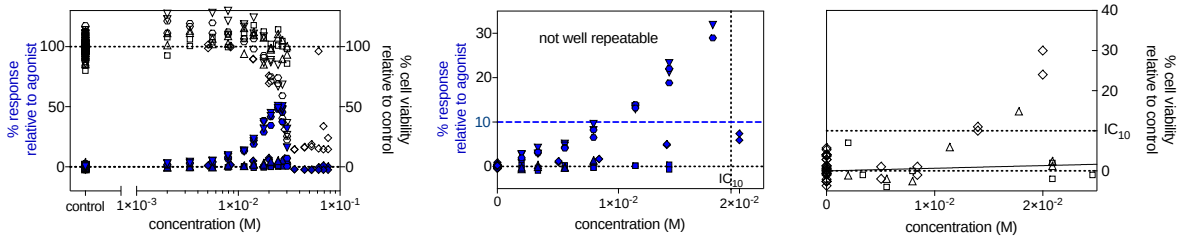
On the left plot are all data plotted on a logarithmic concentration scale with the percent response in relation to the potent agonist 2,3,7,8-Tetrachlorodibenzodioxin represented in blue filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



2-Allylphenol



2-Butoxyethanol



2,4,5-Trichloroaniline

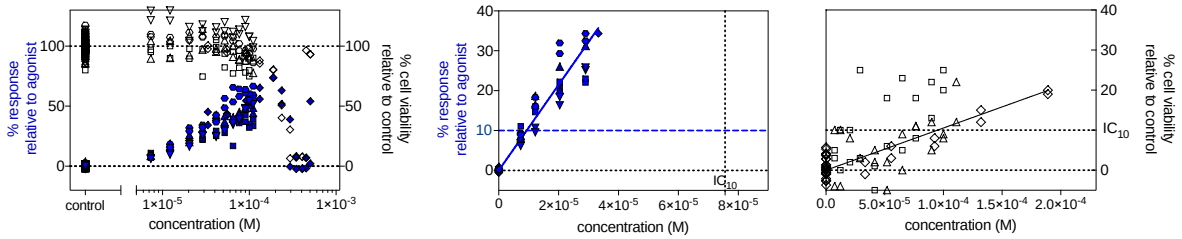
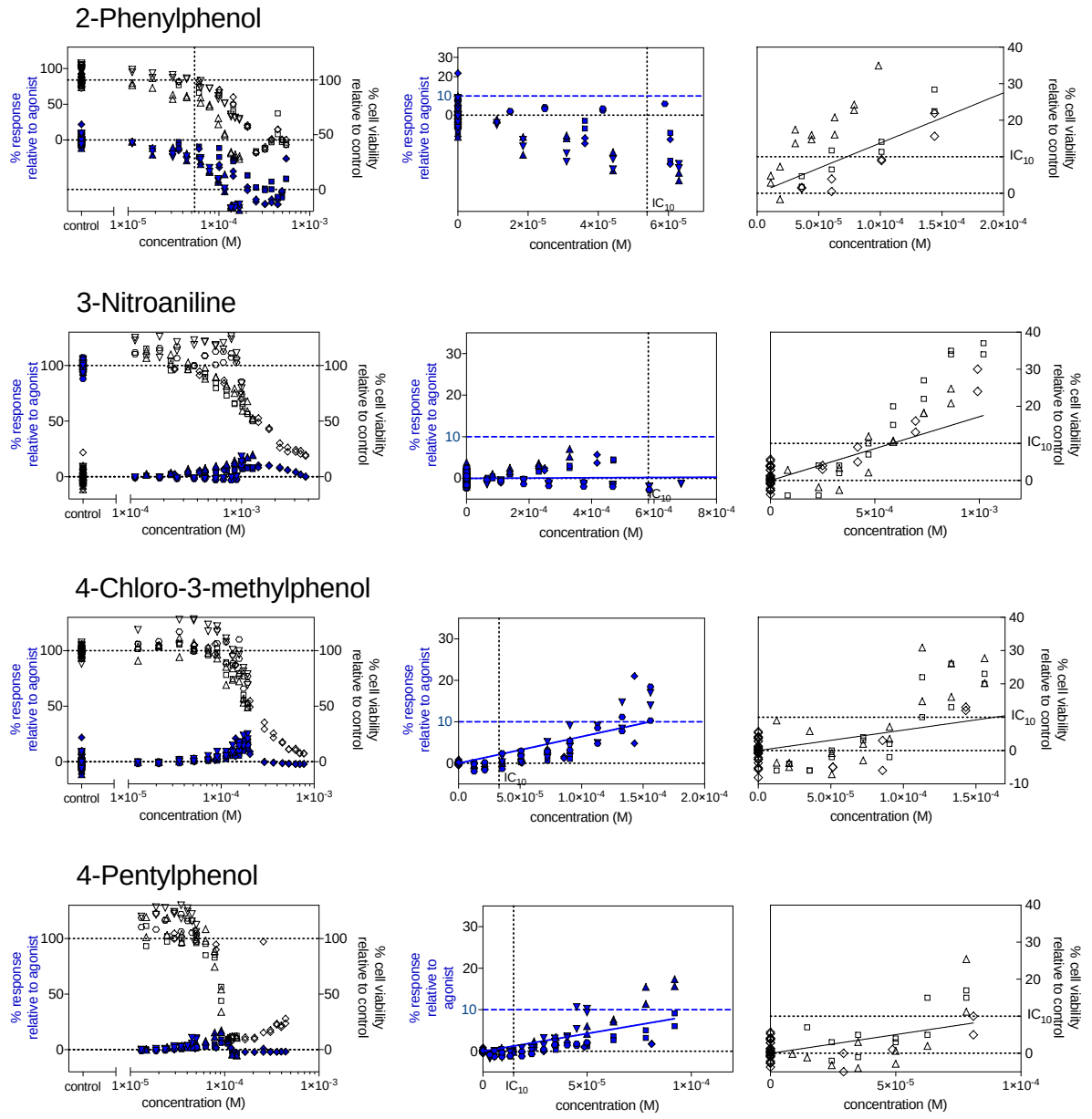


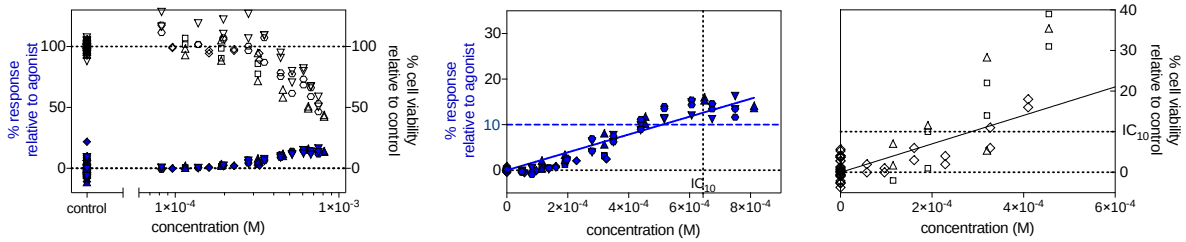
Figure S4. Concentration-cytotoxicity and concentration-effect relationships of the confirmed baseline toxicants in PPARγ-BLA.

On the left plot are all data plotted on a logarithmic concentration scale with the percent response in relation to the potent agonist Rosiglitazone represented in blue filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.

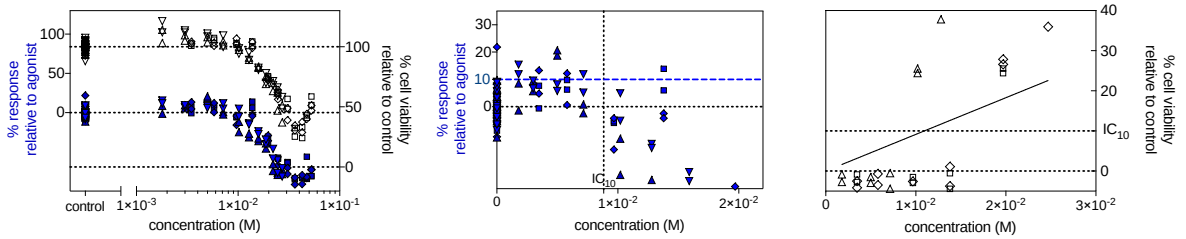


Supplementary Material
Cytotoxicity burst? Differentiating specific from nonspecific effects in reporter gene assays

2-Allylphenol



2-Butoxyethanol



2,4,5-Trichloroaniline

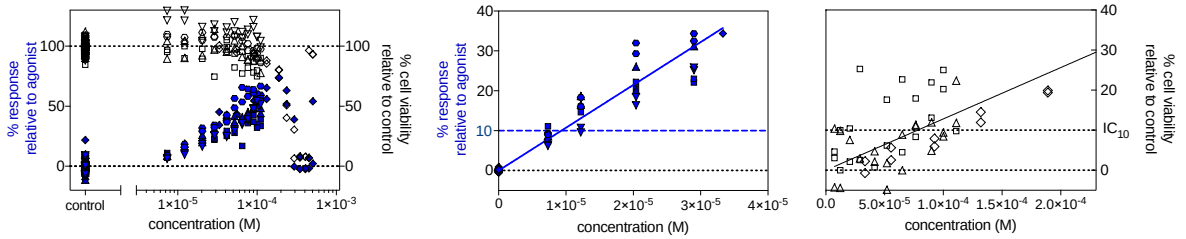
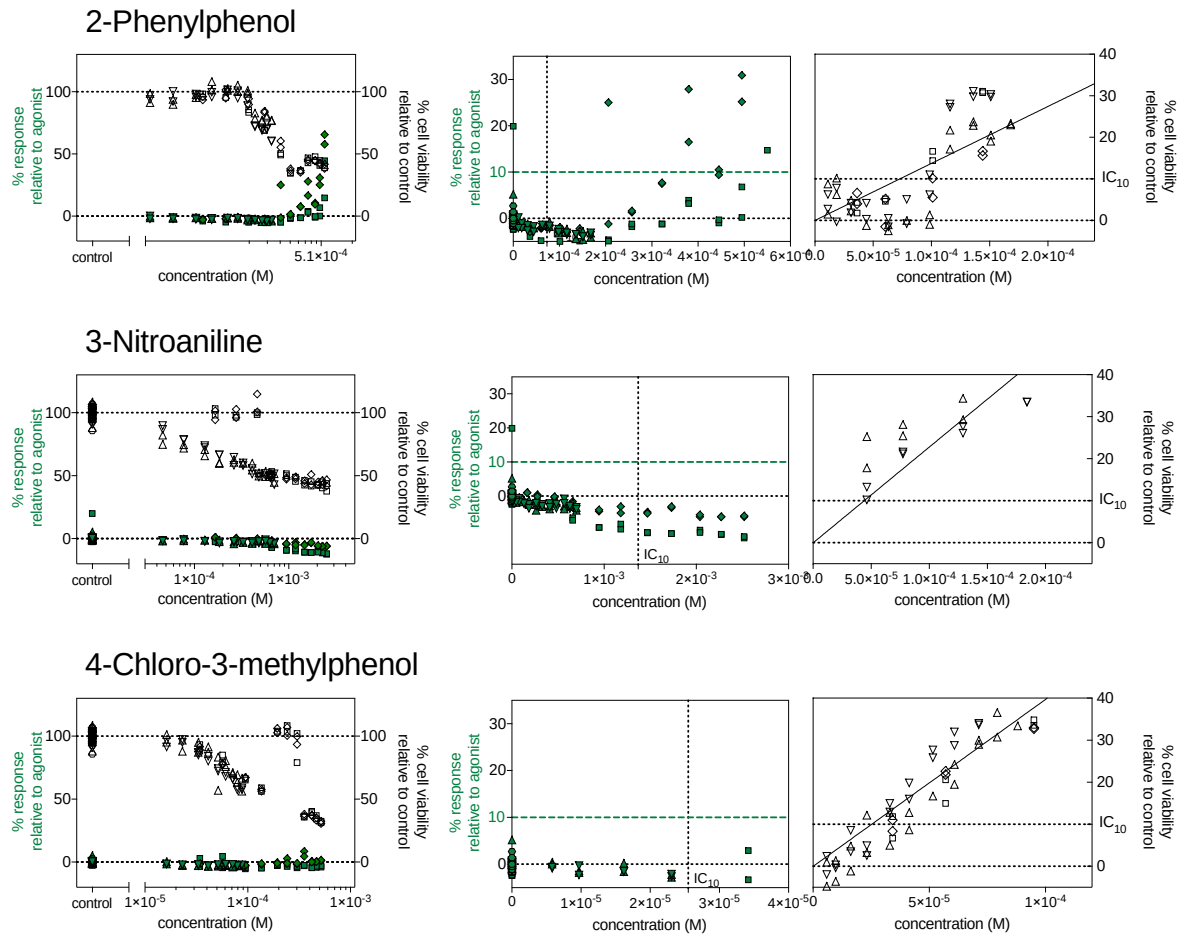


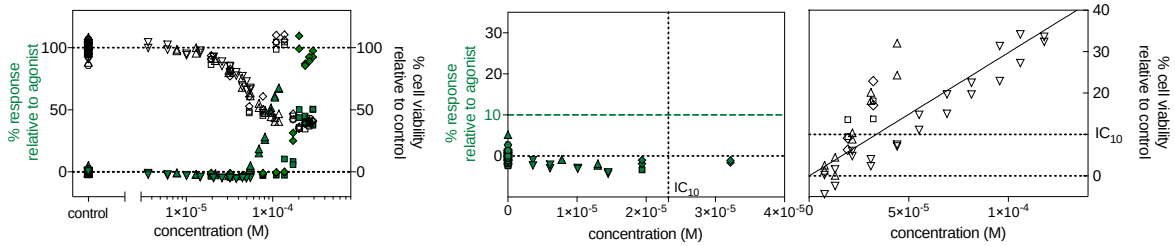
Figure S5. Concentration-cytotoxicity and concentration-effect relationships of the confirmed baseline toxicants in AR-BLA.

On the left plot are all data plotted on a logarithmic concentration scale with the percent response relative to the maximum effect represented in green filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.

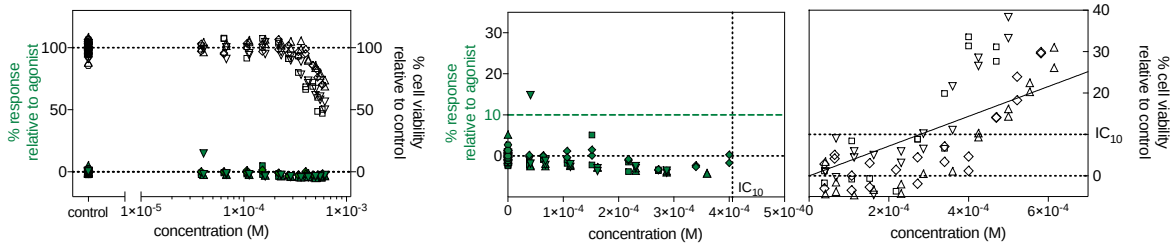


Supplementary Material
Cytotoxicity burst? Differentiating specific from nonspecific effects in reporter gene assays

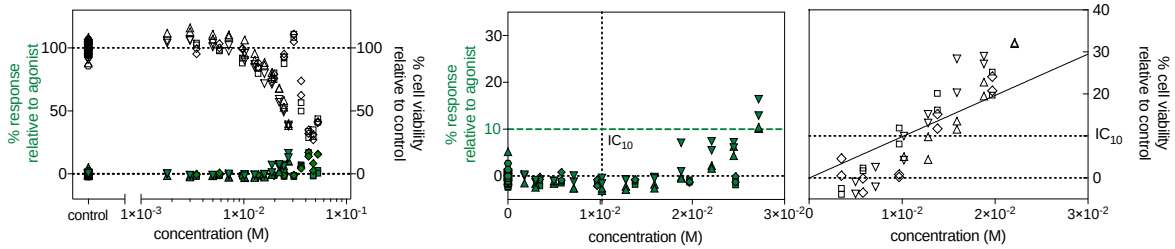
4-Pentylphenol



2-Allylphenol



Butoxyethanol



2,4,5-Trichloroaniline

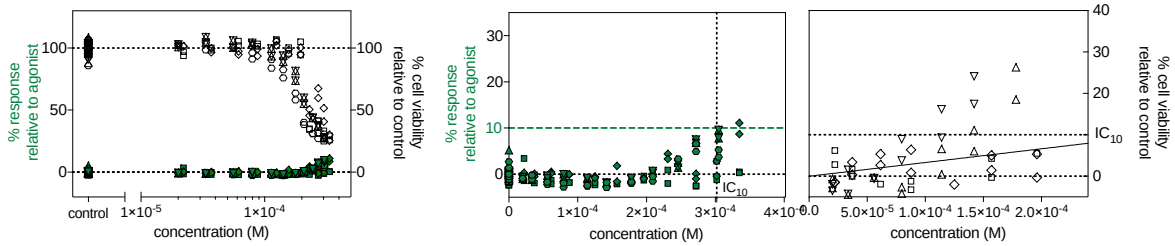
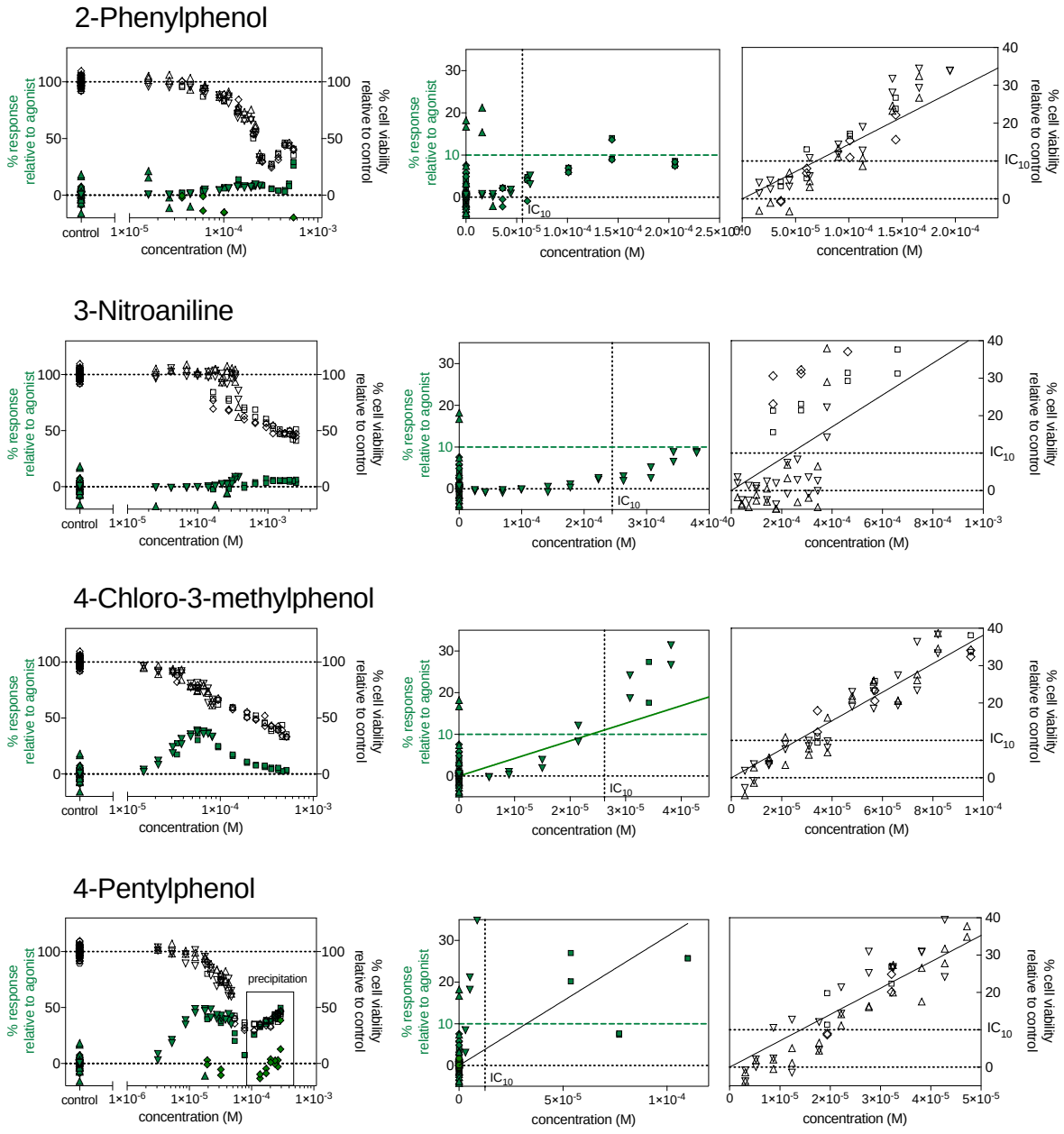
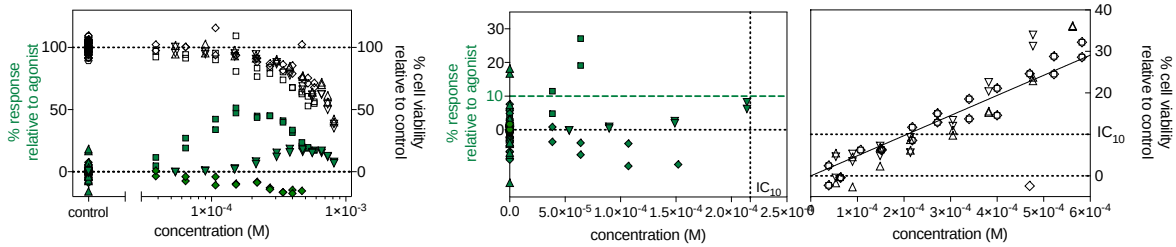


Figure S6. Concentration-cytotoxicity and concentration-effect relationships of the confirmed baseline toxicants in ERα-BLA.

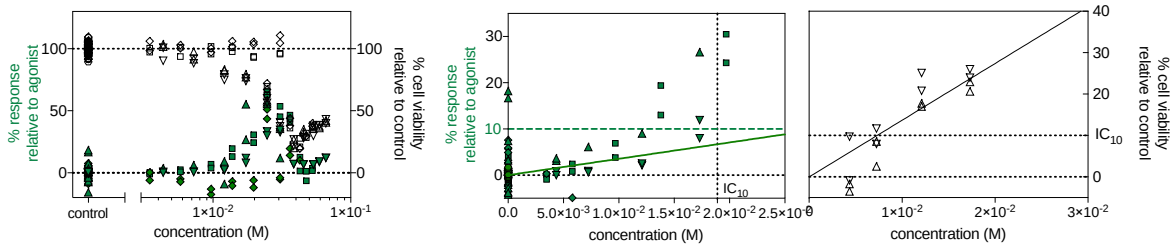
On the left plot are all data plotted on a logarithmic concentration scale with the percent response relative to the maximum effect represented in green filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



2-Allylphenol



2-Butoxyethanol



2,4,5-Tricloroaniline

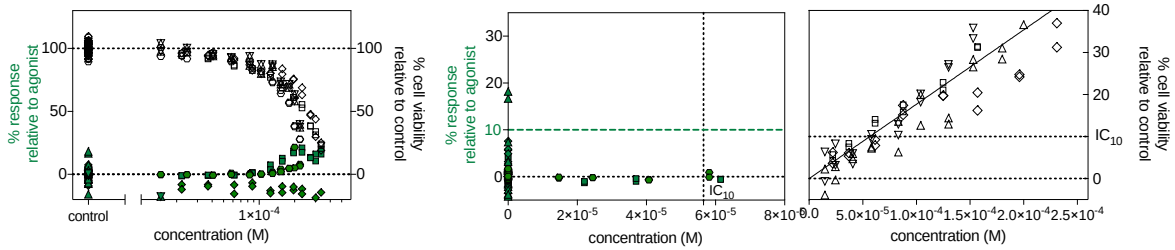
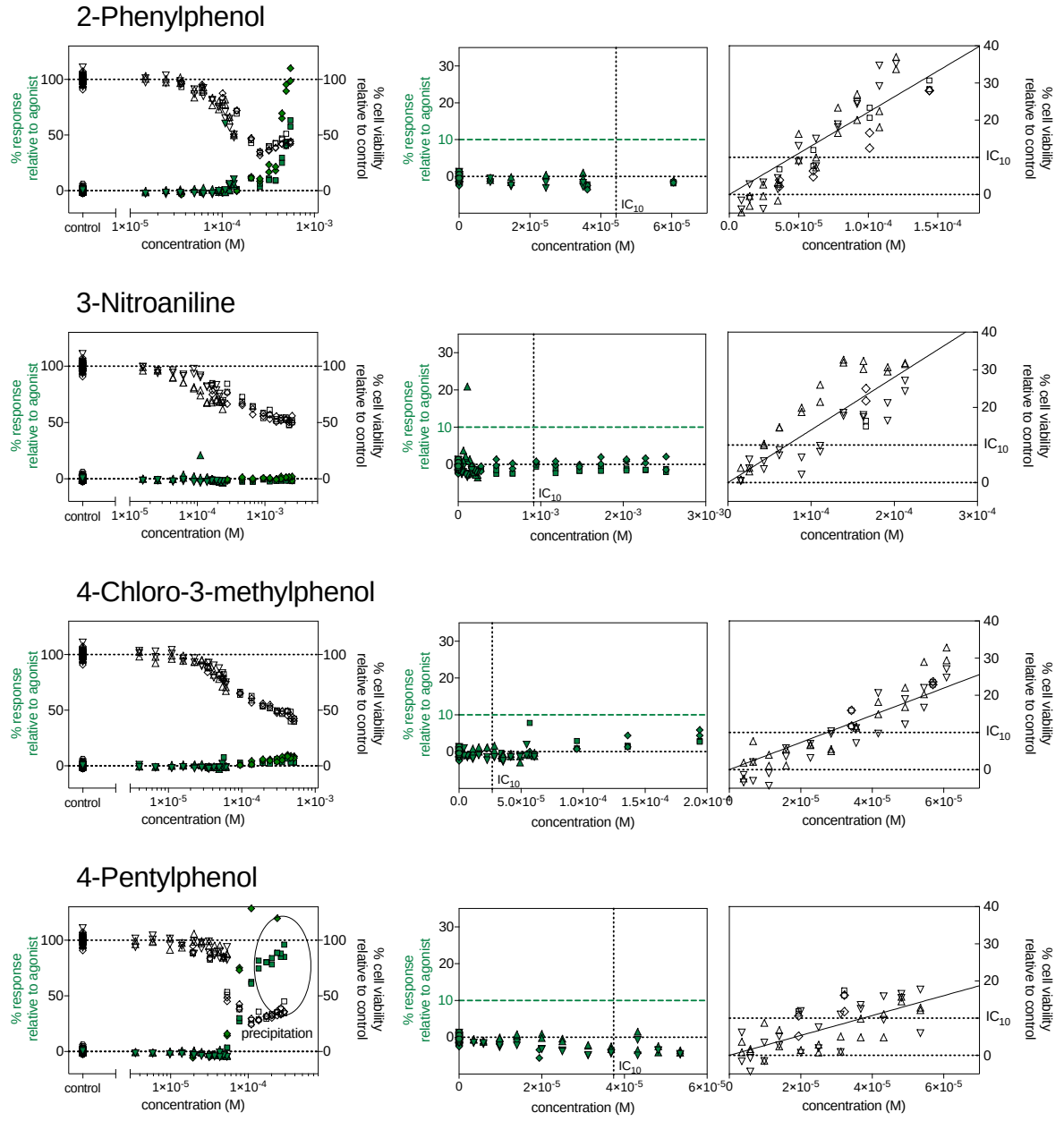


Figure S7. Concentration-cytotoxicity and concentration-effect relationships of the confirmed baseline toxicants in PR-BLA.

On the left plot are all data plotted on a logarithmic concentration scale with the percent response relative to the maximum effect represented in green filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



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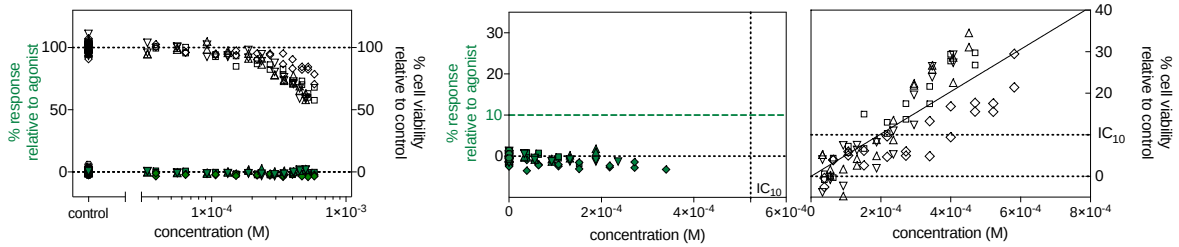
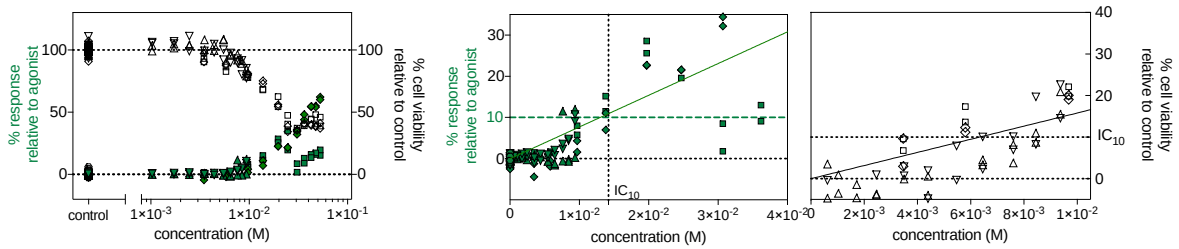


Butoxyethanol



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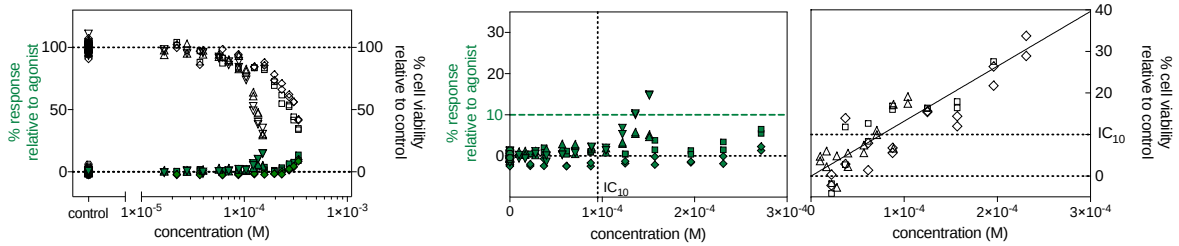
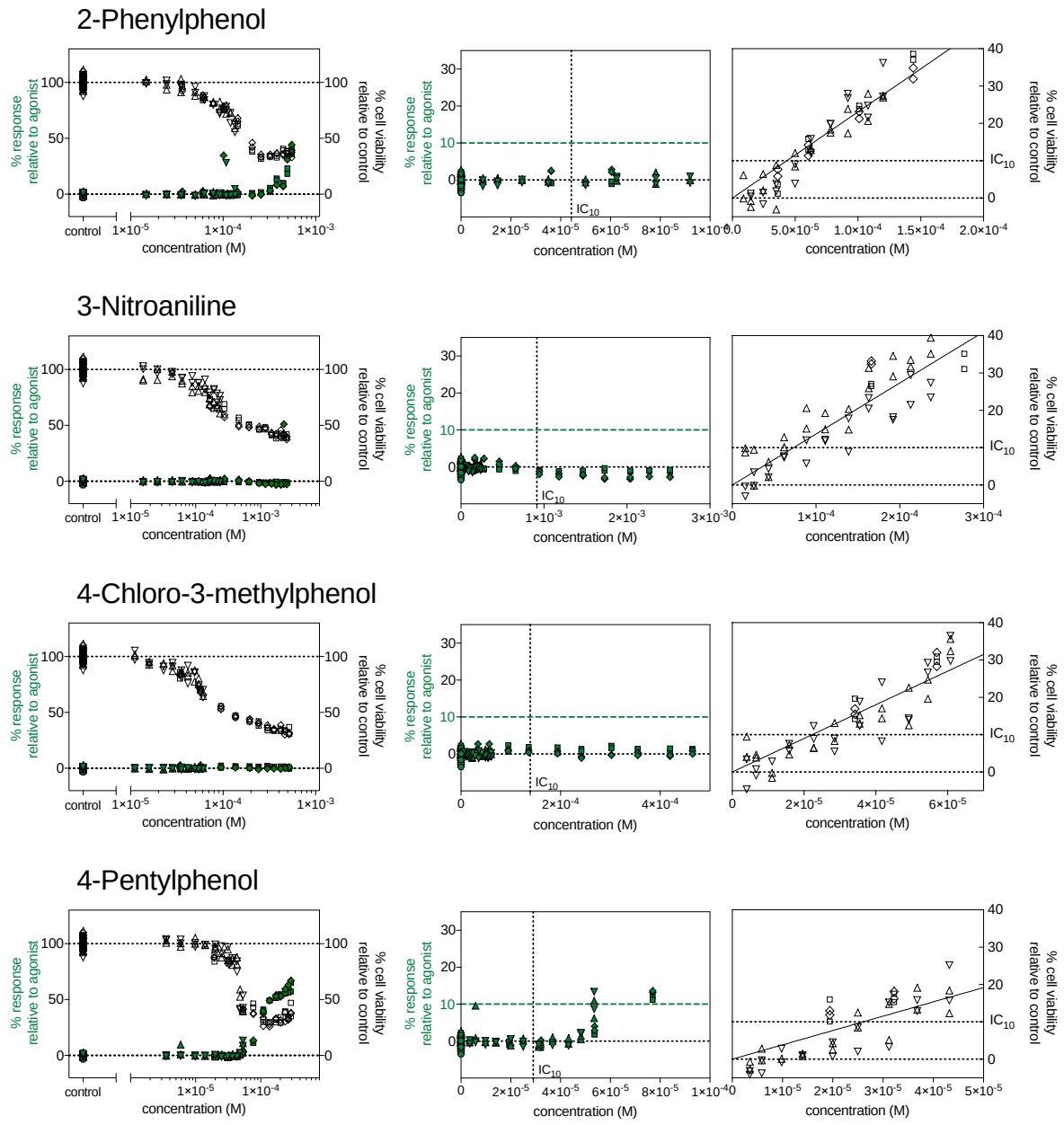


Figure S8. Concentration-cytotoxicity and concentration-effect relationships of the confirmed baseline toxicants in GR-BLA.

On the left plot are all data plotted on a logarithmic concentration scale with the percent response relative to the maximum effect represented in green filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



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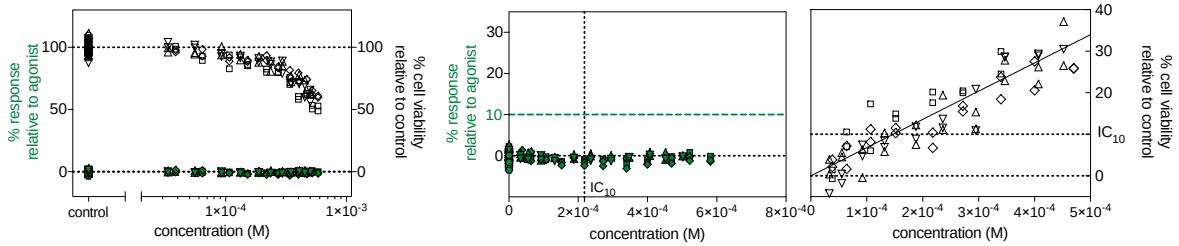

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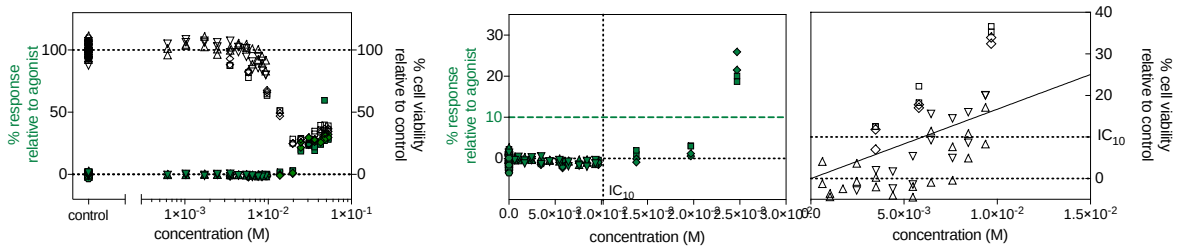

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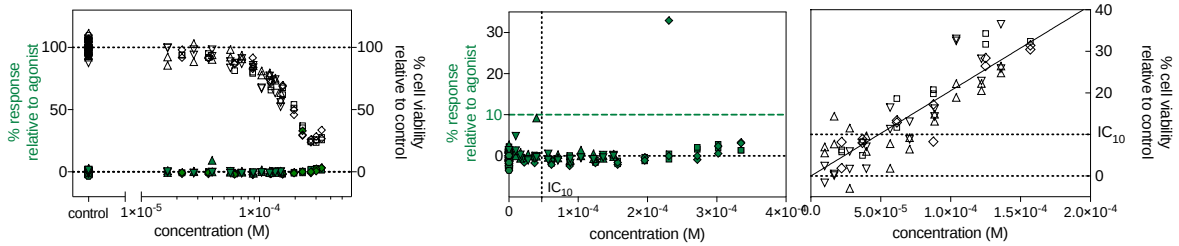
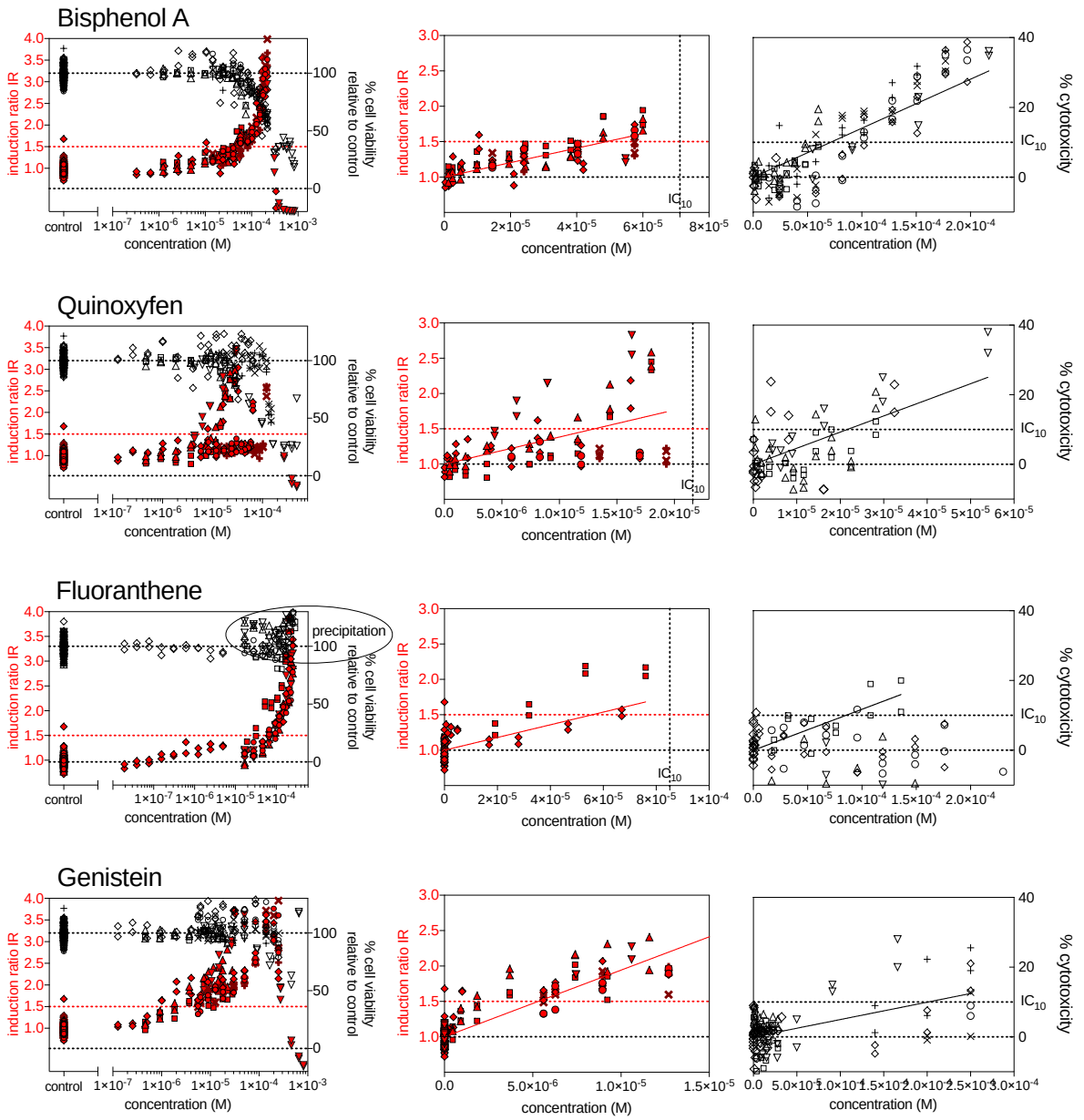


Figure S9. Concentration-cytotoxicity and concentration-effect relationships of the environmental chemicals in AREc32.

On the left plot are all data plotted on a logarithmic concentration scale with the induction ratio of AREc32 represented in red filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the $EC_{IR1.5}$ is derived (IR 1.5 effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



Supplementary Material
Cytotoxicity burst? Differentiating specific from nonspecific effects in reporter gene assays

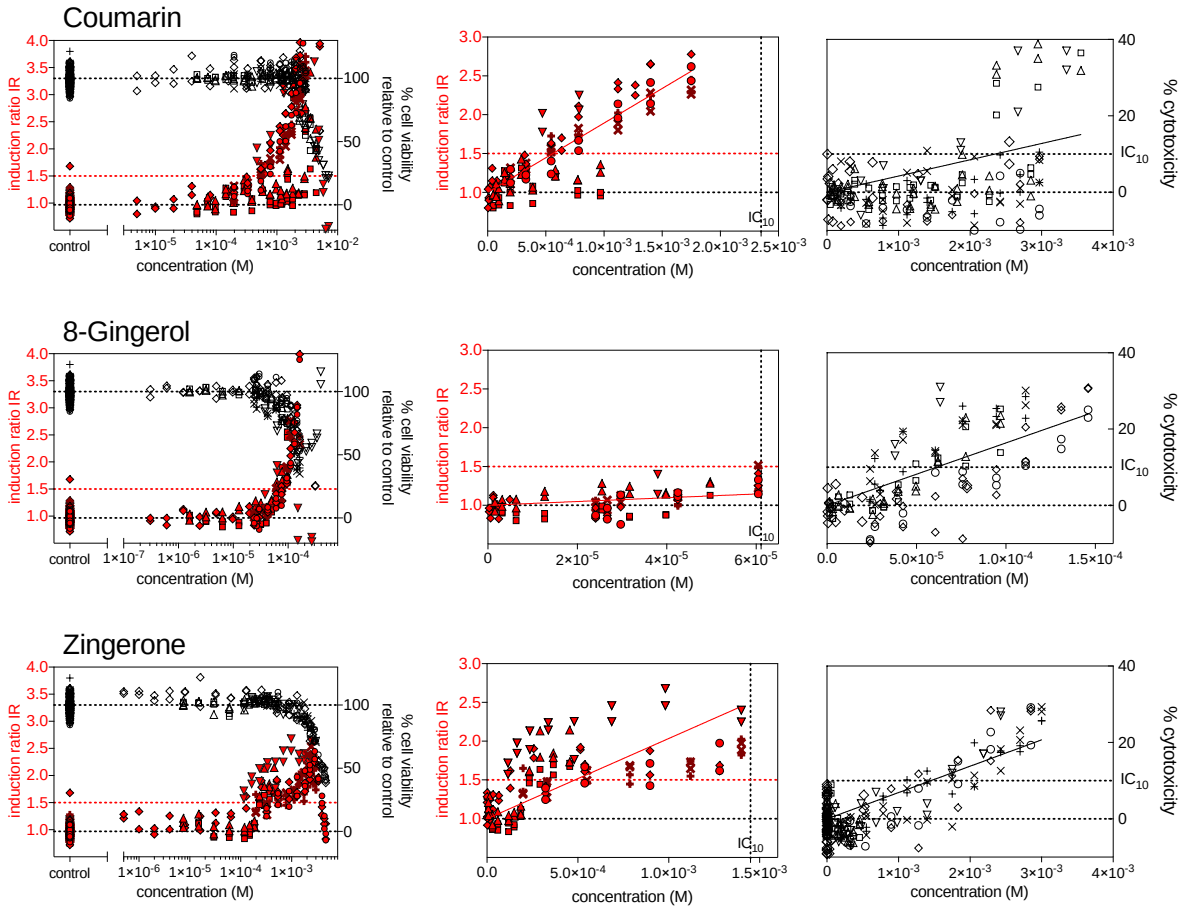
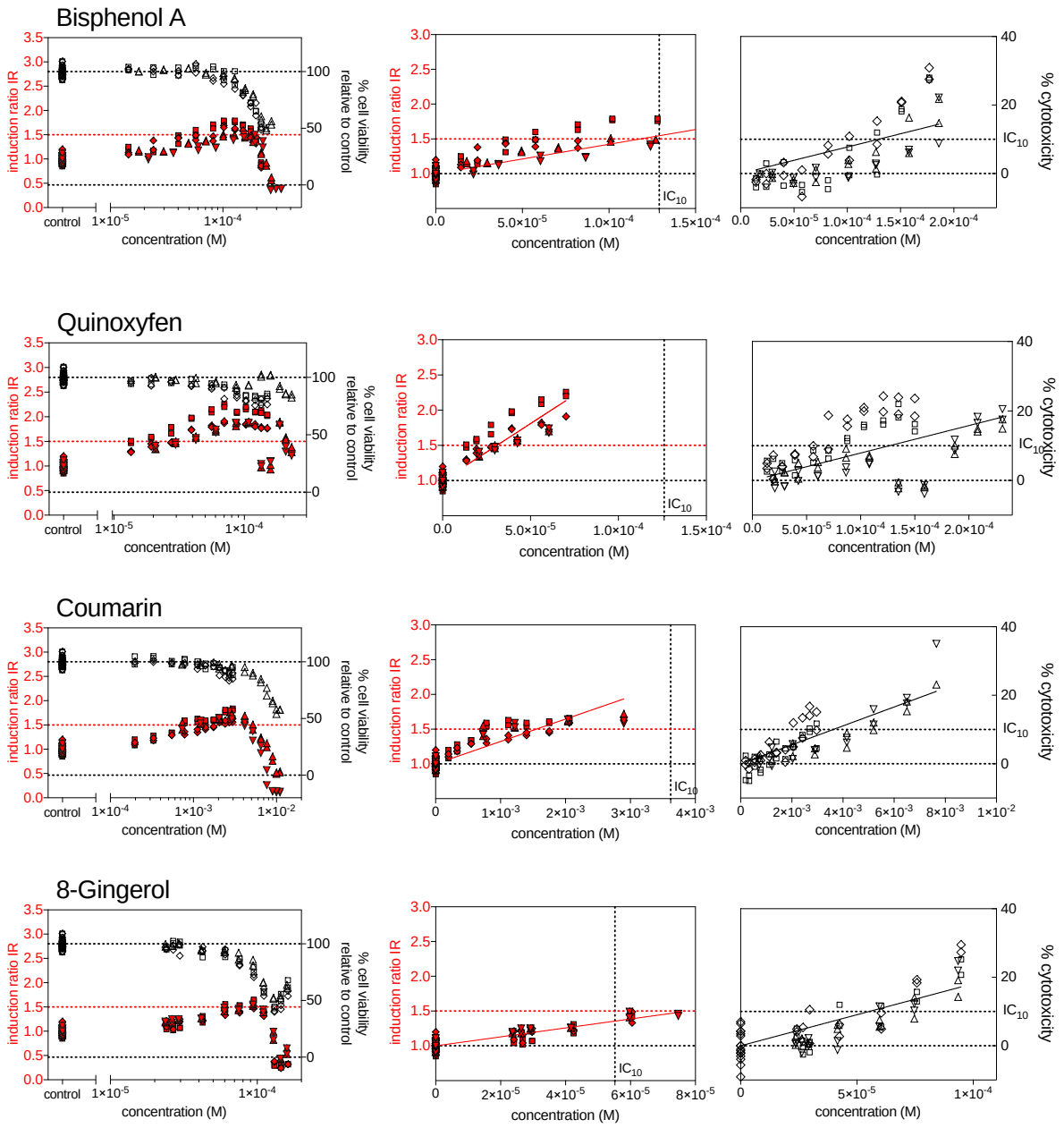


Figure S10. Concentration-cytotoxicity and concentration-effect relationships of the environmental chemicals in ARE-BLA.

On the left plot are all data plotted on a logarithmic concentration scale with the induction ratio of ARE-BLA represented in red filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the $EC_{IR1.5}$ is derived (IR 1.5 effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



Supplementary Material
Cytotoxicity burst? Differentiating specific from nonspecific effects in reporter gene assays

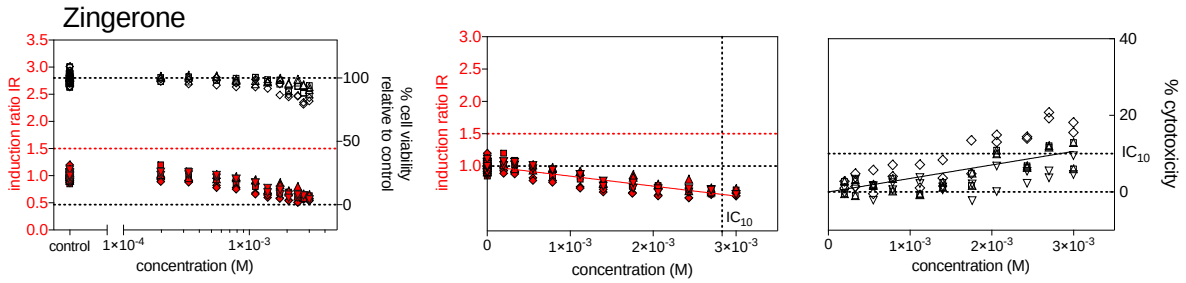
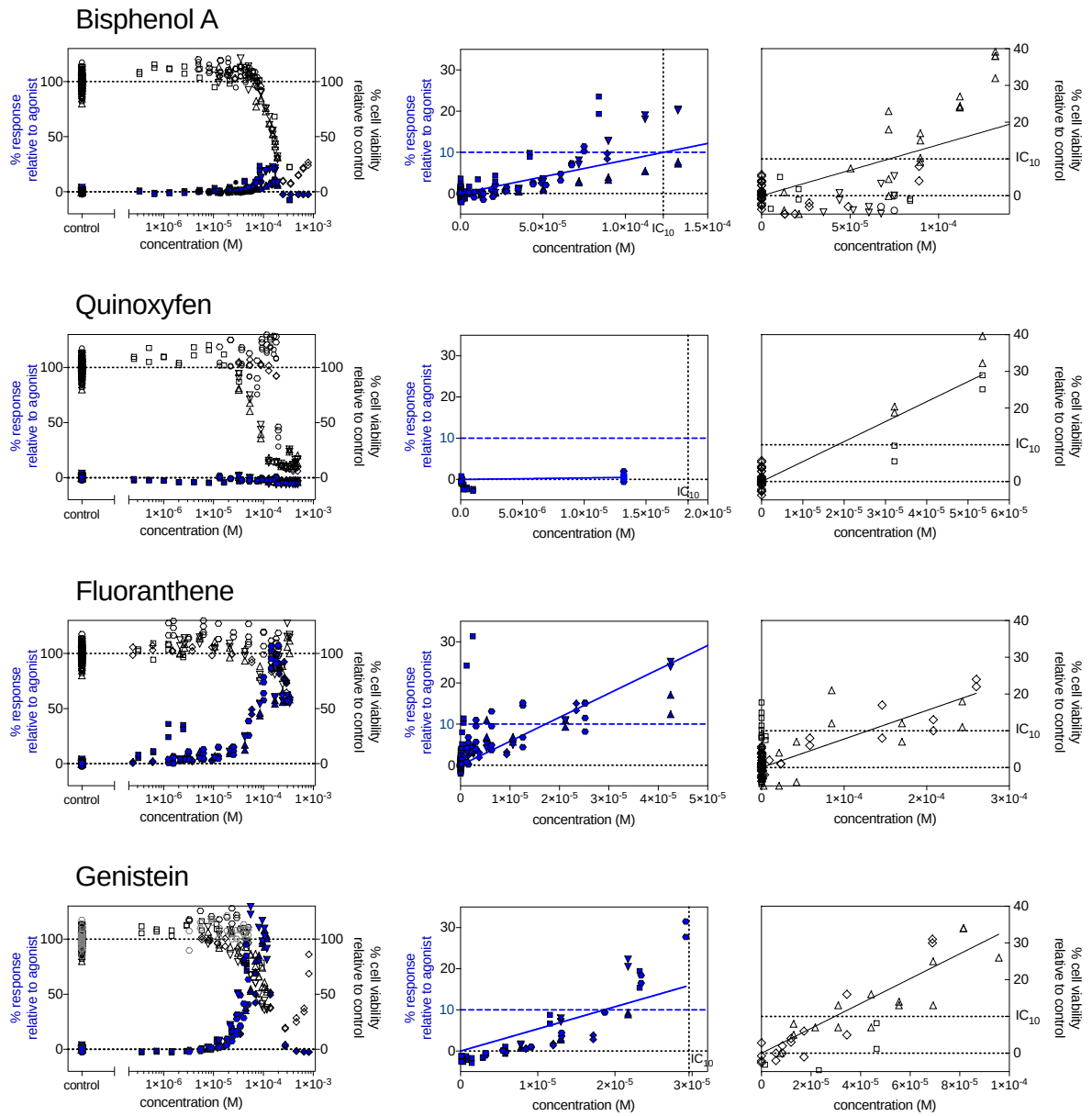


Figure S11. Concentration-cytotoxicity and concentration-effect relationships of the environmental chemicals in AhR-CALUX.

On the left plot are all data plotted on a logarithmic concentration scale with the percent response in relation to the potent agonist 2,3,7,8-Tetrachlorodibenzodioxin represented in blue filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



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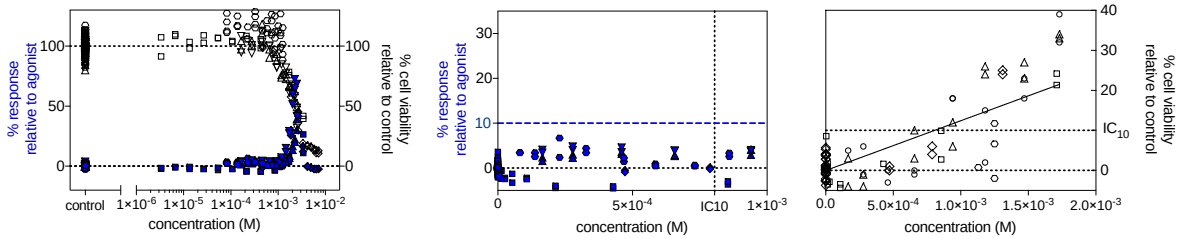
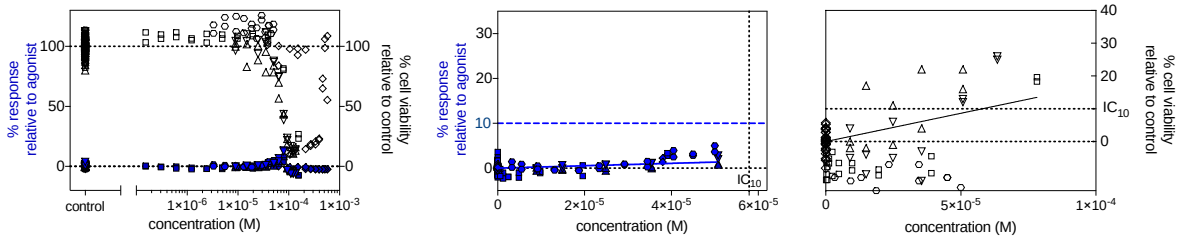


8-Gingerol



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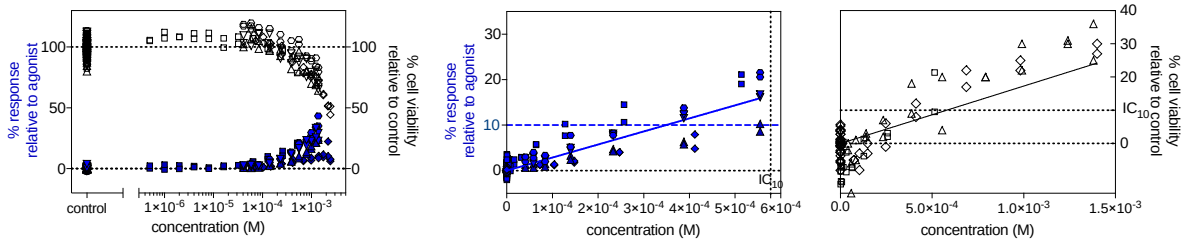
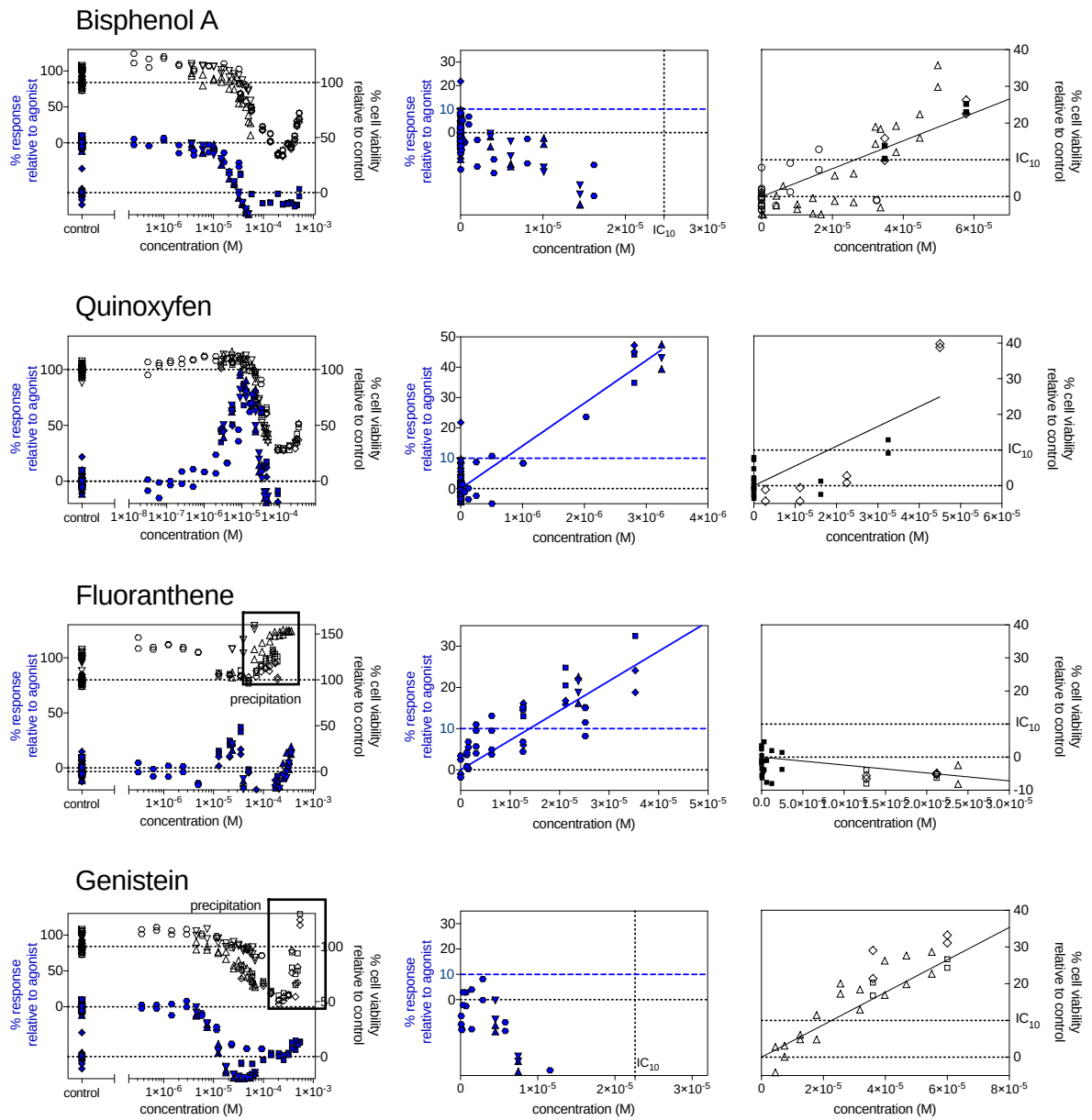


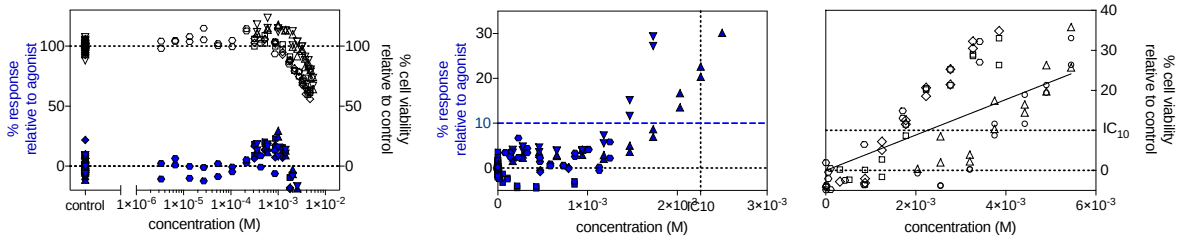
Figure S12. Concentration-cytotoxicity and concentration-effect relationships of the environmental chemicals in PPARγ-BLA.

On the left plot are all data plotted on a logarithmic concentration scale with the percent response in relation to the potent agonist Rosiglitazone represented in blue filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



Supplementary Material
Cytotoxicity burst? Differentiating specific from nonspecific effects in reporter gene assays

Coumarin



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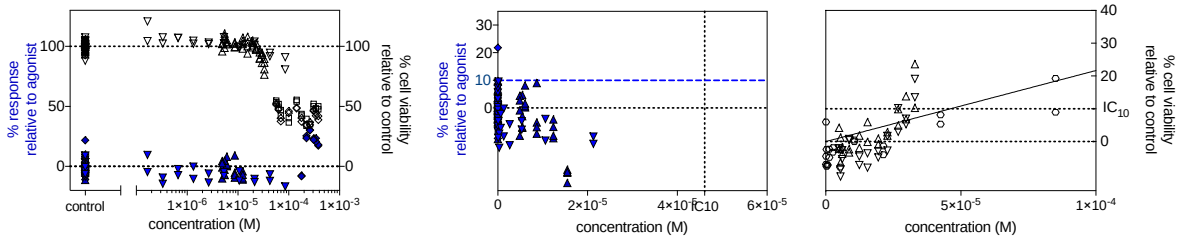

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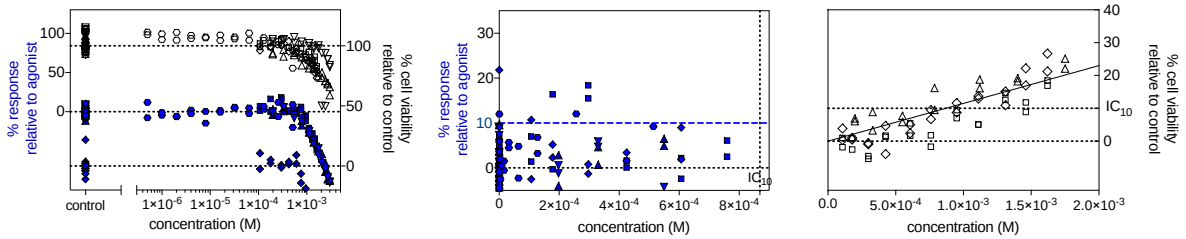
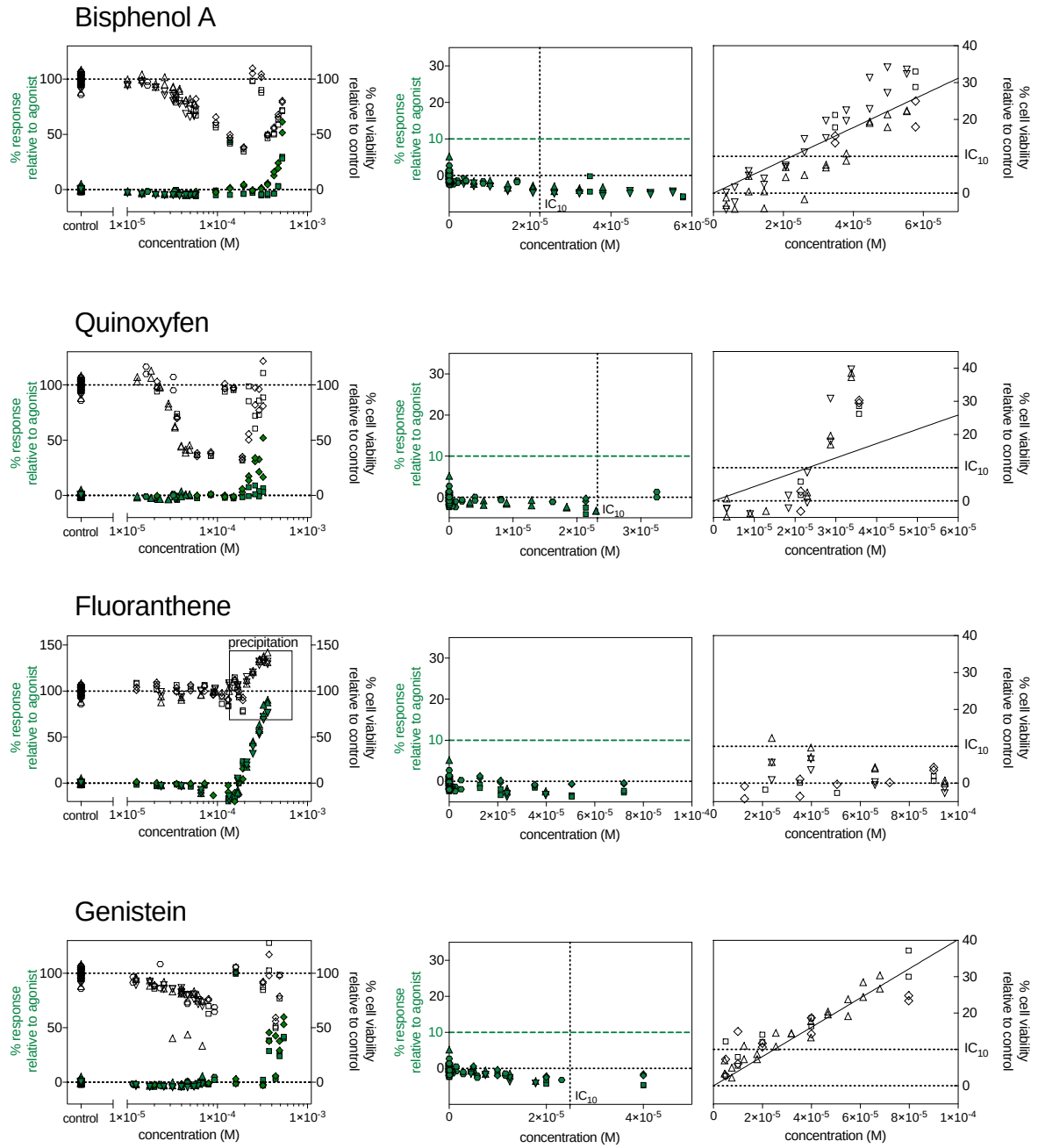


Figure S13. Concentration-cytotoxicity and concentration-effect relationships of the environmental chemicals in AR-BLA.

On the left plot are all data plotted on a logarithmic concentration scale with the percent response relative to the maximum effect represented in green filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



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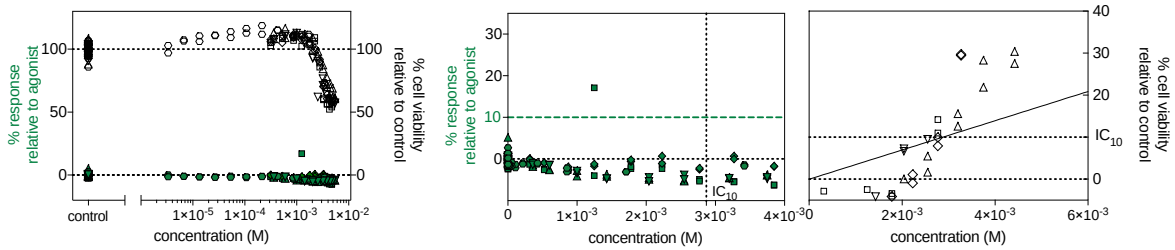

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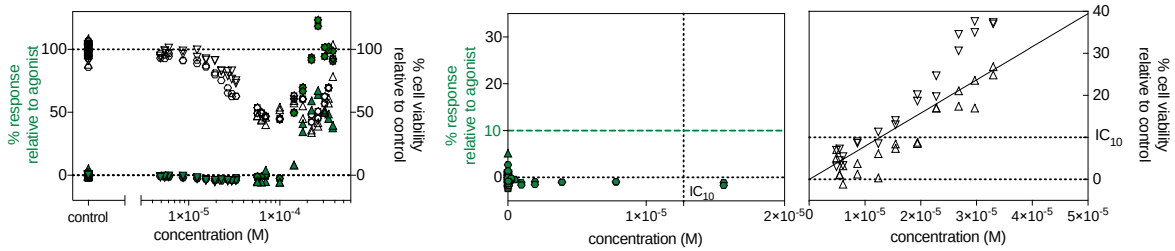

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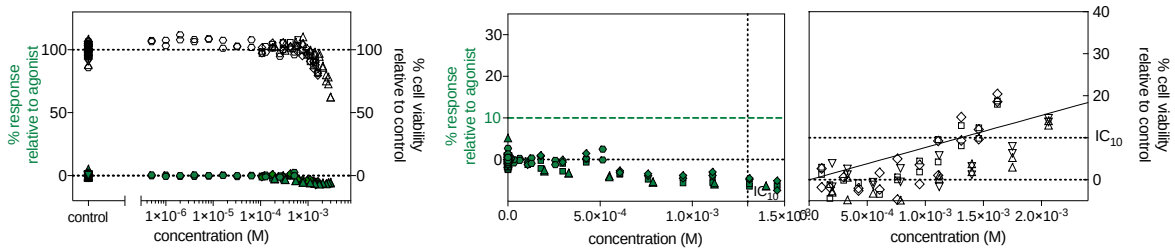
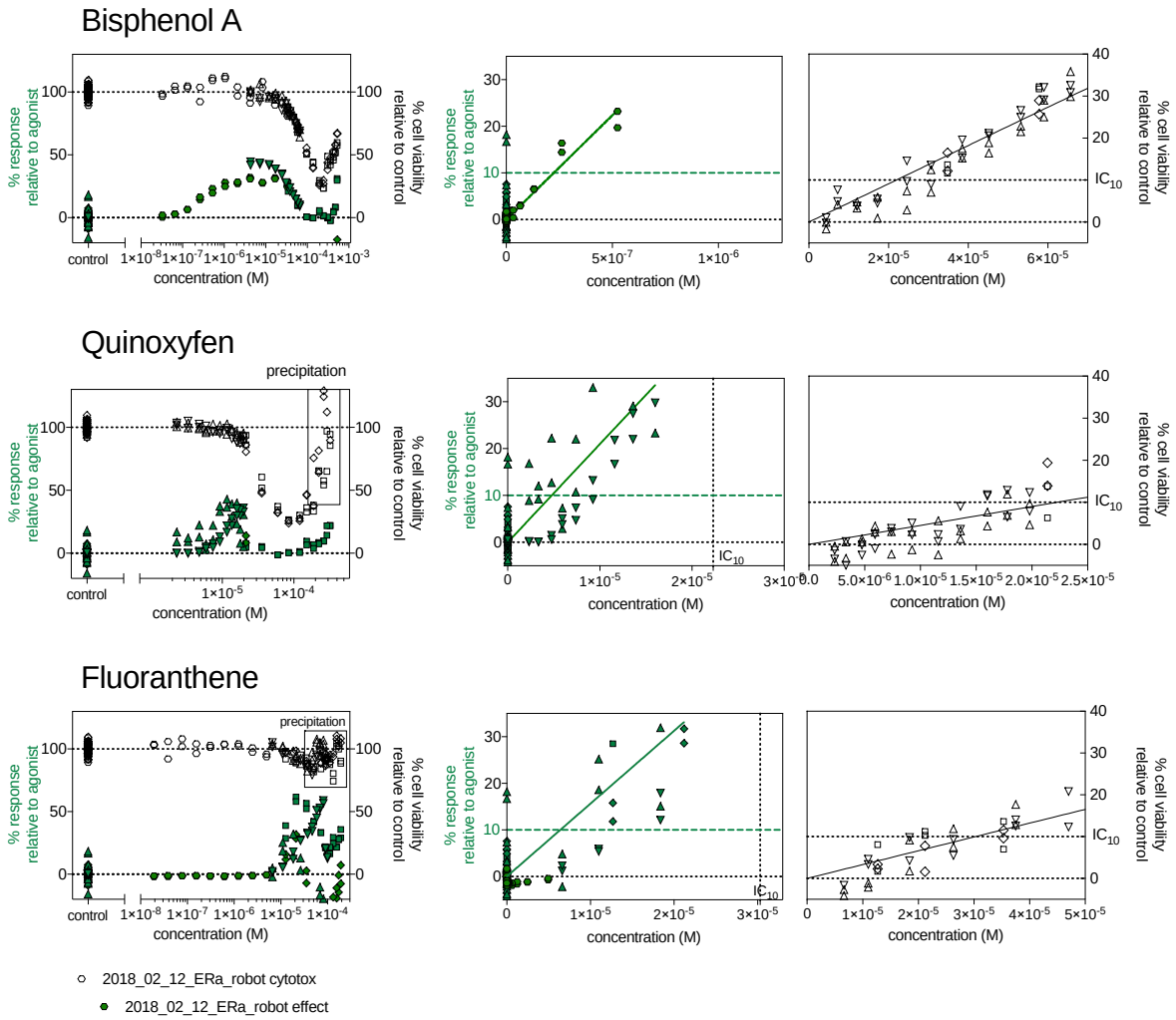


Figure S14. Concentration-cytotoxicity and concentration-effect relationships of the environmental chemicals in ERα-BLA.

On the left plot are all data plotted on a logarithmic concentration scale with the percent response relative to the maximum effect represented in green filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



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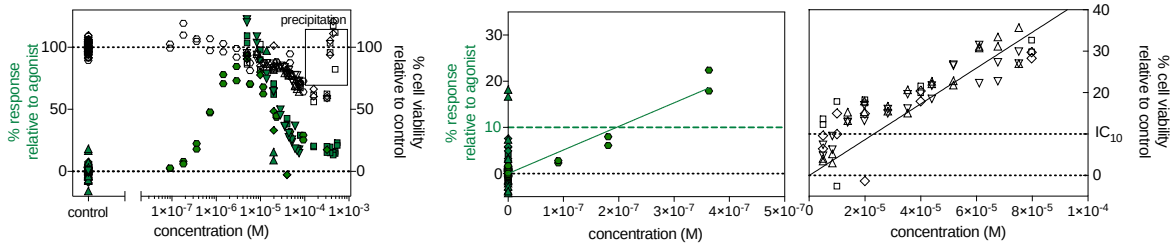

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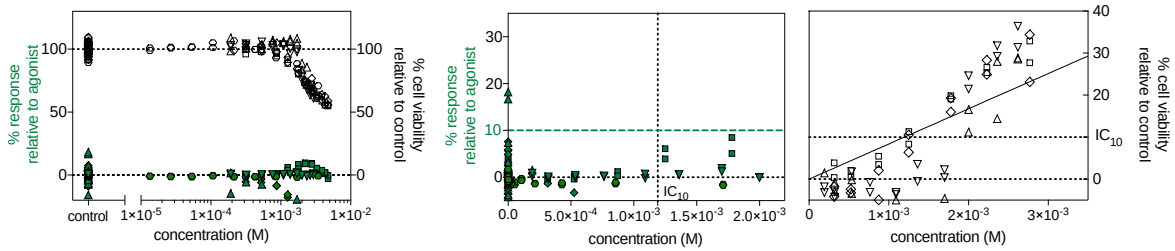

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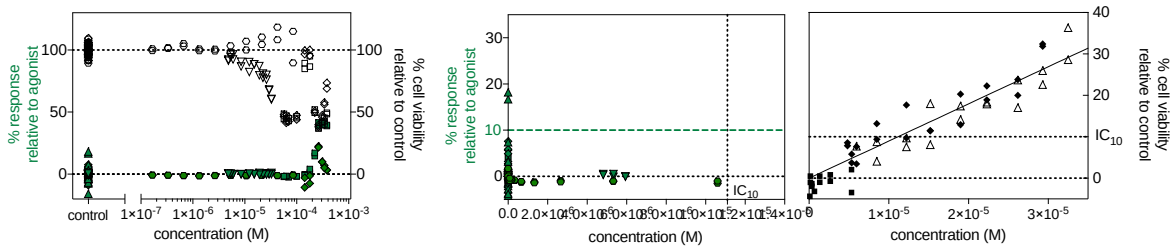

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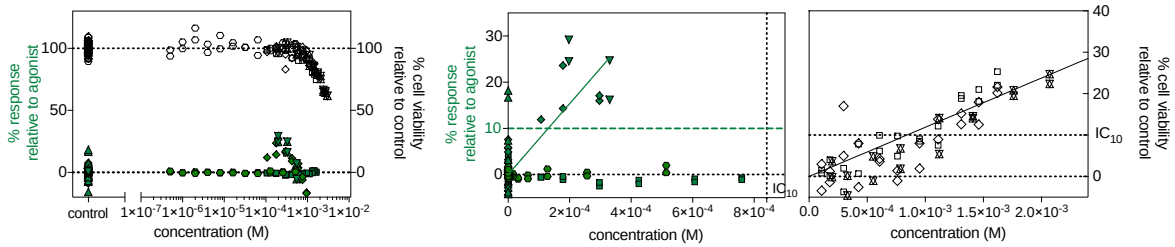
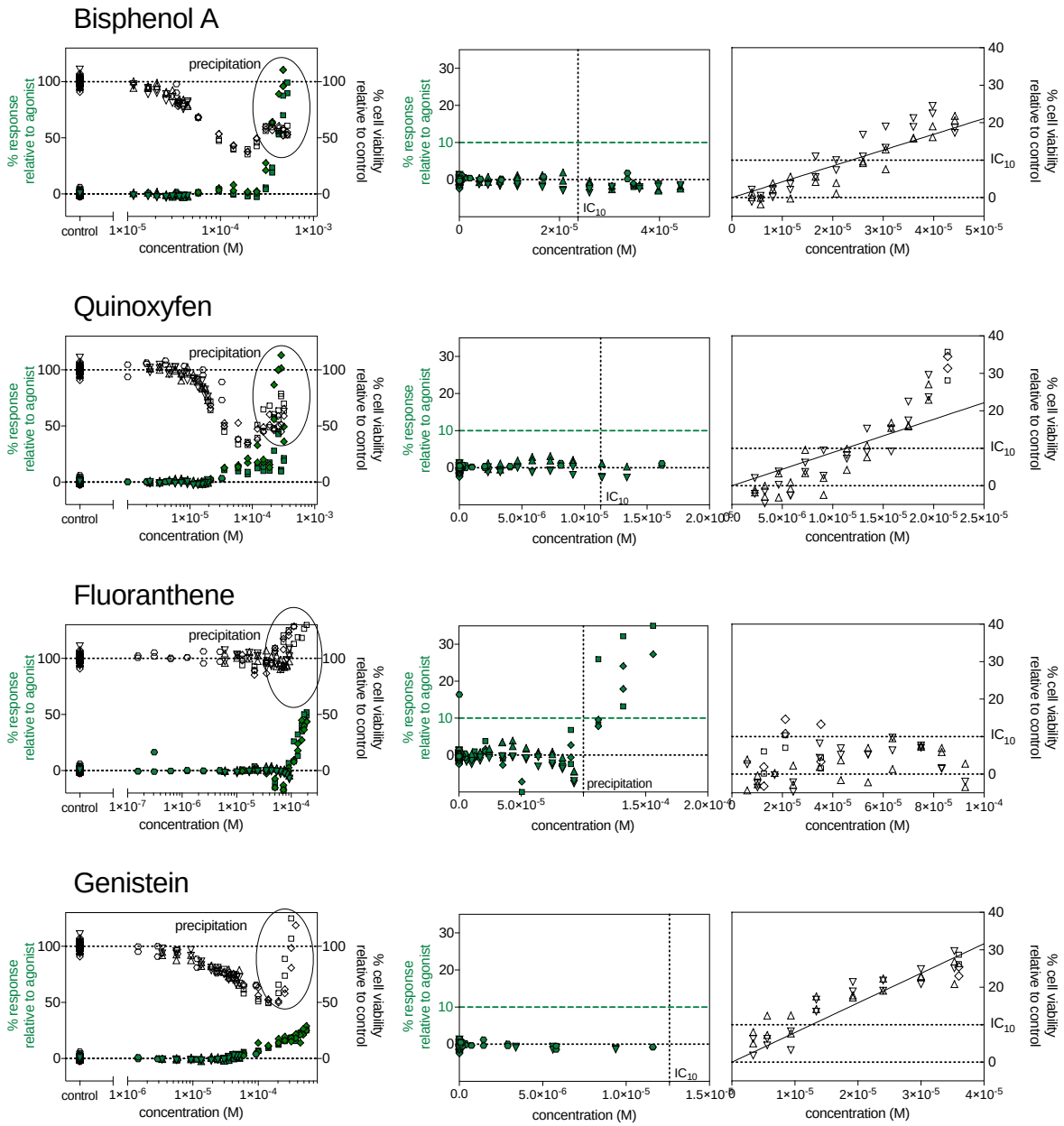


Figure S15. Concentration-cytotoxicity and concentration-effect relationships of the environmental chemicals in PR-BLA.

On the left plot are all data plotted on a logarithmic concentration scale with the percent response relative to the maximum effect represented in green filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



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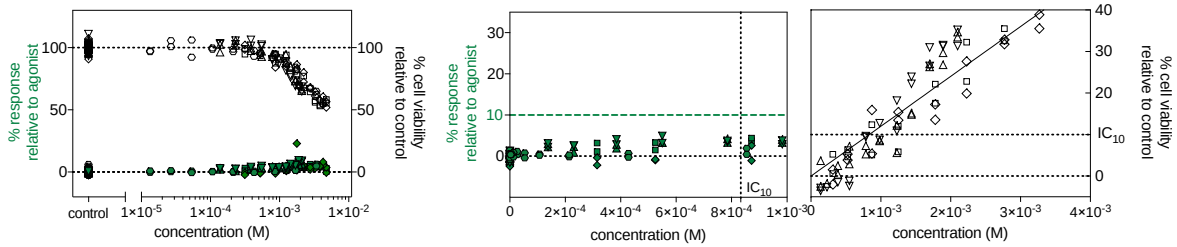

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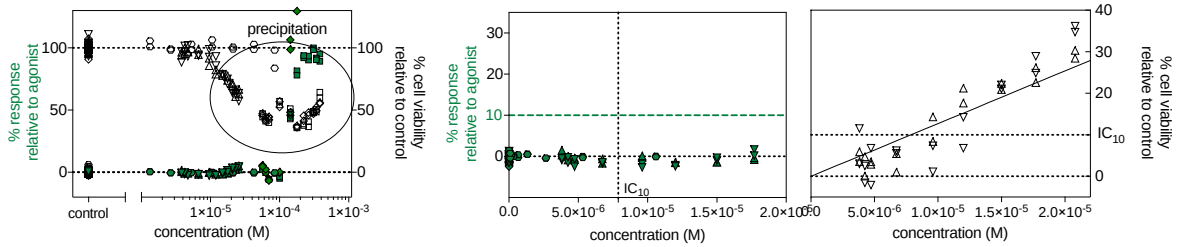

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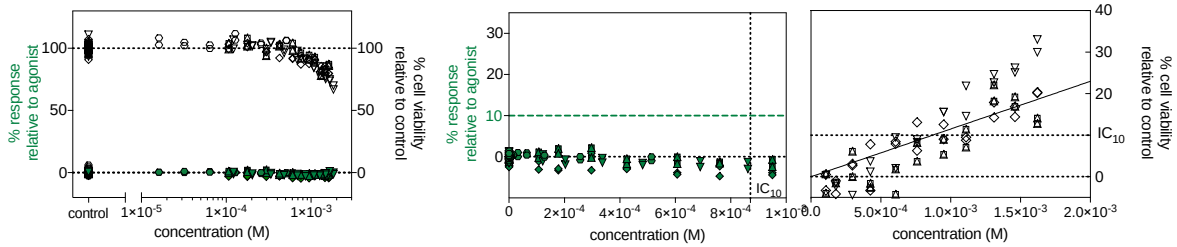
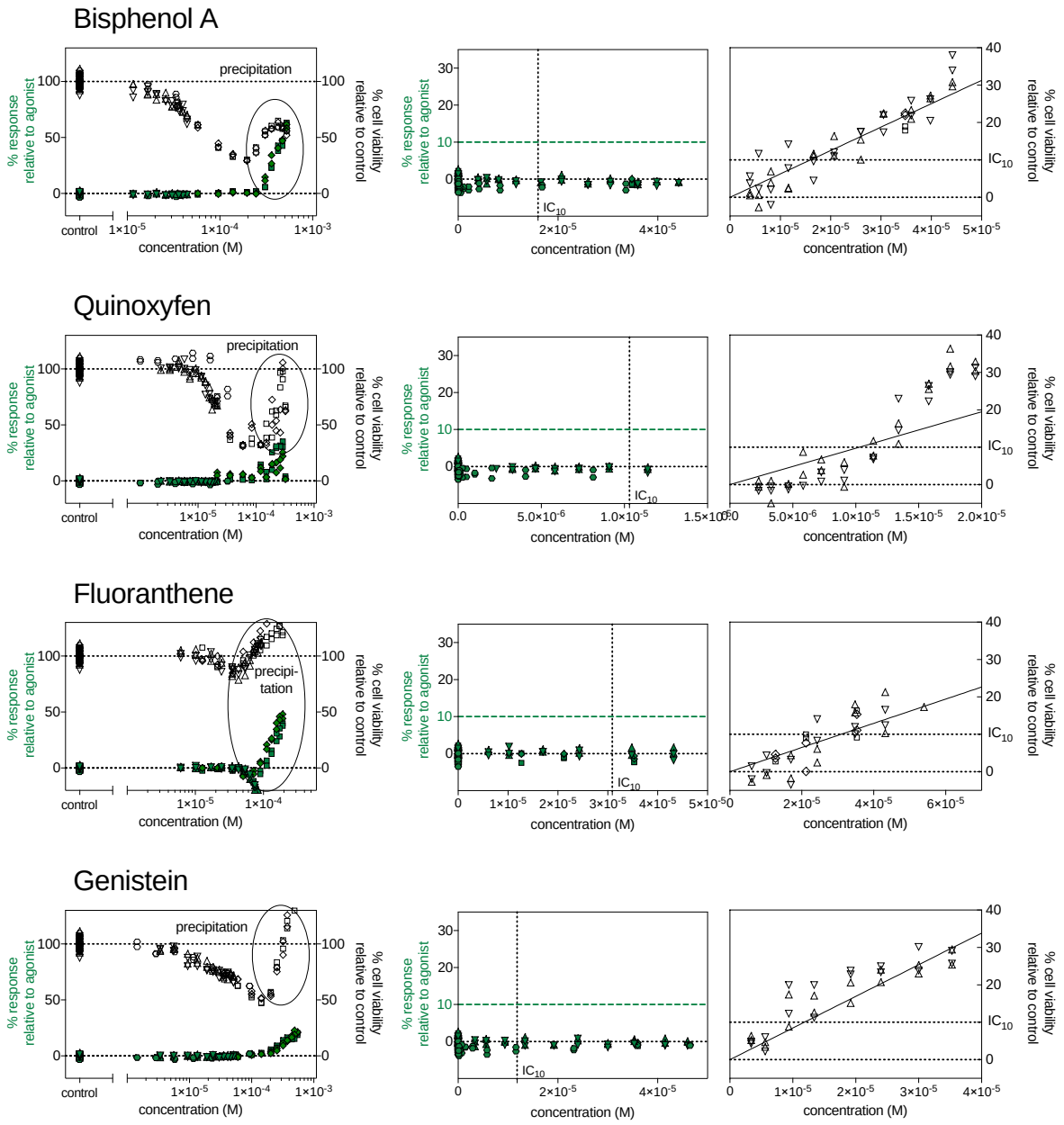


Figure S16. Concentration-cytotoxicity and concentration-effect relationships of the environmental chemicals in GR-BLA.

On the left plot are all data plotted on a logarithmic concentration scale with the percent response relative to the maximum effect represented in green filled symbols (left y-axis) and the cytotoxicity in empty black-rimmed symbols (right y-axis). Different symbols refer to different independent experiments. On the right are only experimental cytotoxicity data depicted that are in the linear portion of the concentration-cytotoxicity relationship (solid line) up to 40% cytotoxicity (=100% - % cell viability), from which the IC_{10} is derived (10% effect level marked by a dotted line). In the middle plot are only experimental effect data in the linear portion of the concentration-effect relationship, from which the EC_{10} is derived (10% effect level marked by a dotted horizontal line); IC_{10}, which is normally the cut-off for evaluation of reporter gene activation, marked by a vertical dotted line.



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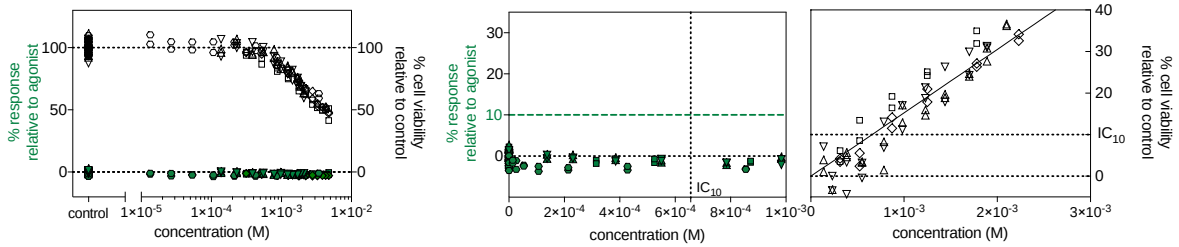

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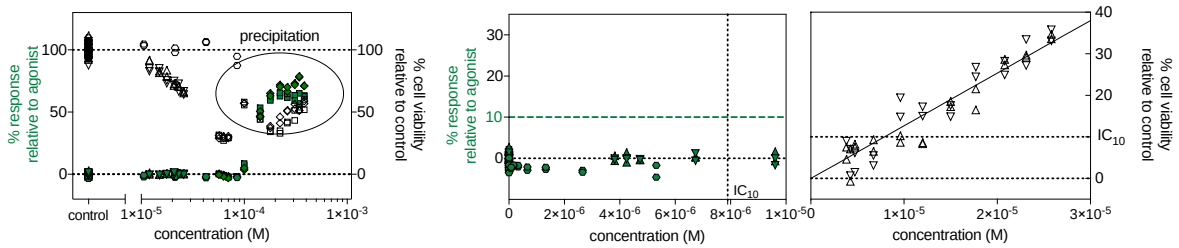

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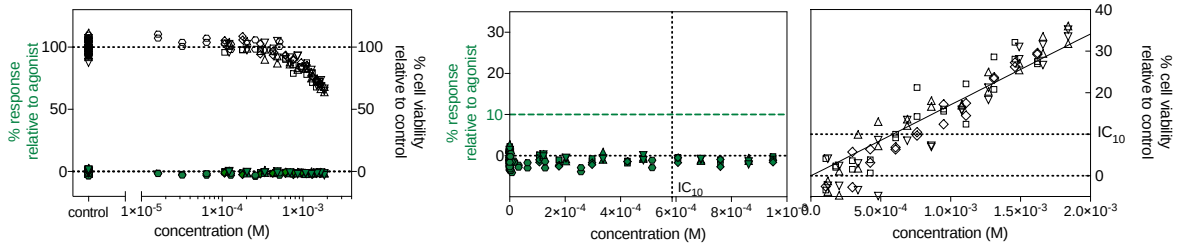


Figure S17. Cytotoxicity of the additional seven chemicals in relation to the Quantitative Structure Activity Relationships (QSAR) for baseline toxicity for all cell lines.

The colored symbols with chemicals' names are the additional chemicals. The black diamonds are the experimental data of the baseline toxicants from (Escher et al. 2019), the solid lines correspond to the best fit. The QSARs equations from (Escher et al. 2019) are reprinted in Table 1. Abbreviations: AhR, arylhydrocarbon receptor; AR, androgen receptor; ARE, Antioxidant response element; BLA, GeneBLAzer reporter gene cell line, CALUX, Chemical Activated LUciferase gene eXpression; ER, estrogen receptor; GR, glucocorticoid receptor, PPARγ, peroxisome proliferator activated receptor gamma; PR, progesterone receptor.

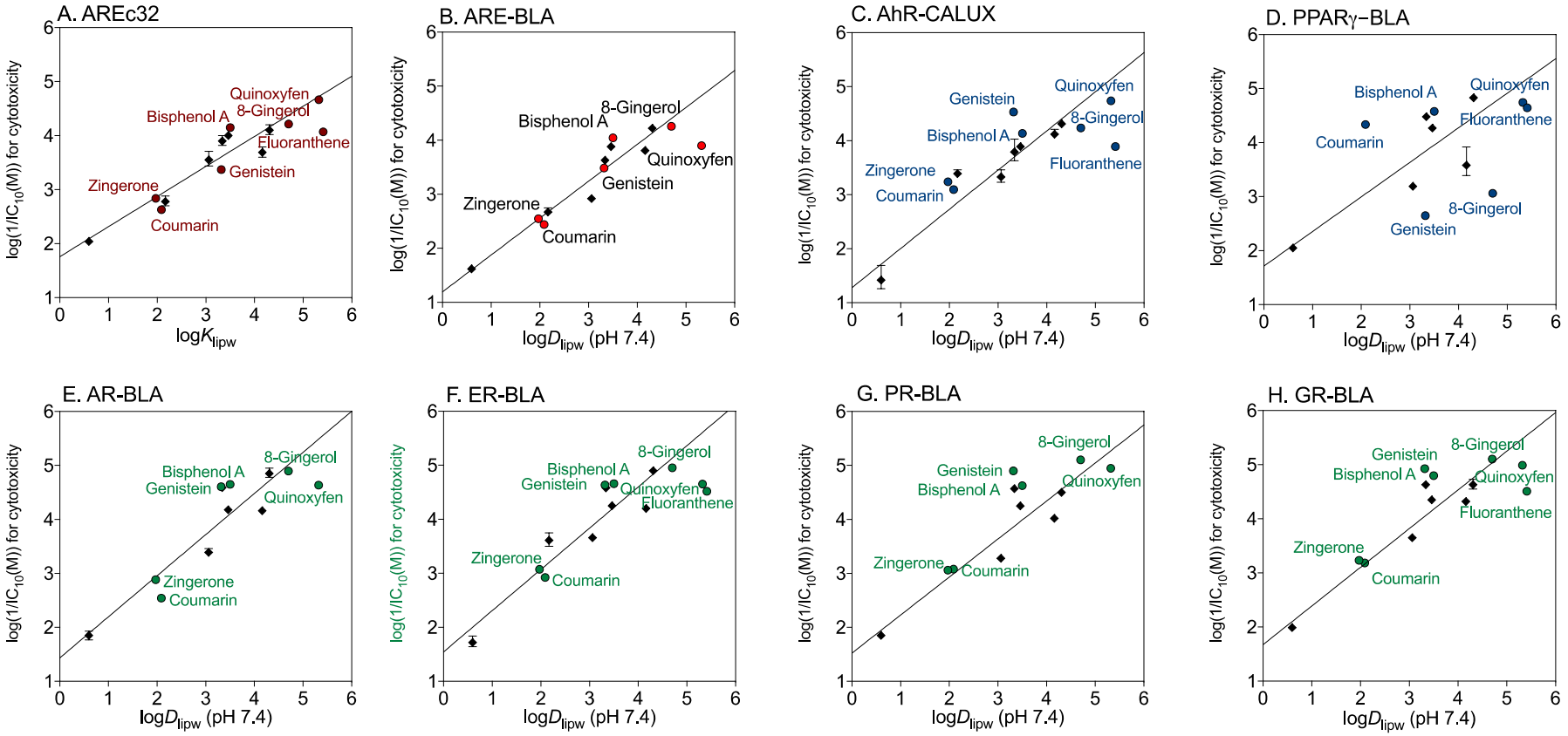


Figure S18. Workflow for the analysis of the Tox21 data.

Abbreviations: $D_{lip/w}$ (pH 7.4), ionization-corrected liposome-water distribution ratio; EC_{10}, effect concentration, leading to 10% effect; IC_{10}, inhibitory concentration, leading to 10% reduction in cell viability; $K_{lip/w}$, liposome-water partition constant; QSAR, Quantitative Structure Activity Relationship; $SR_{cytotoxicity}$, Specificity ratio in relation to experimental cytotoxicity; $SR_{baseline}$, Specificity ratio in relation to baseline cytotoxicity.

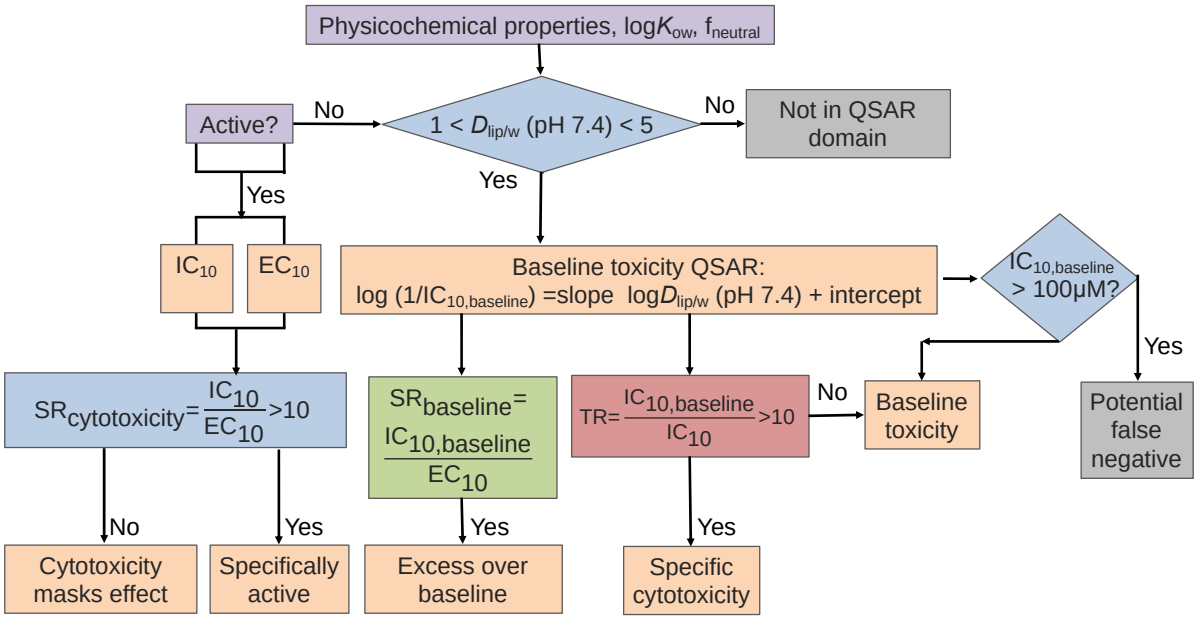


Figure S19. Cytotoxicity of the seven environmental chemicals (Bisphenol A, Quinoxyfen, Fluoranthene, Genistein, Coumarin, 8-Gingerol, Zingerone) in relation to the QSARs for baseline toxicity for all cell lines.

The colored symbols with chemicals' names are the additional chemicals. The black diamonds are the experimental data of the baseline toxicants from (Escher et al. 2019), the solid lines correspond to the best fit. The QSARs equations from (Escher et al. 2019) are reprinted in Table 1.

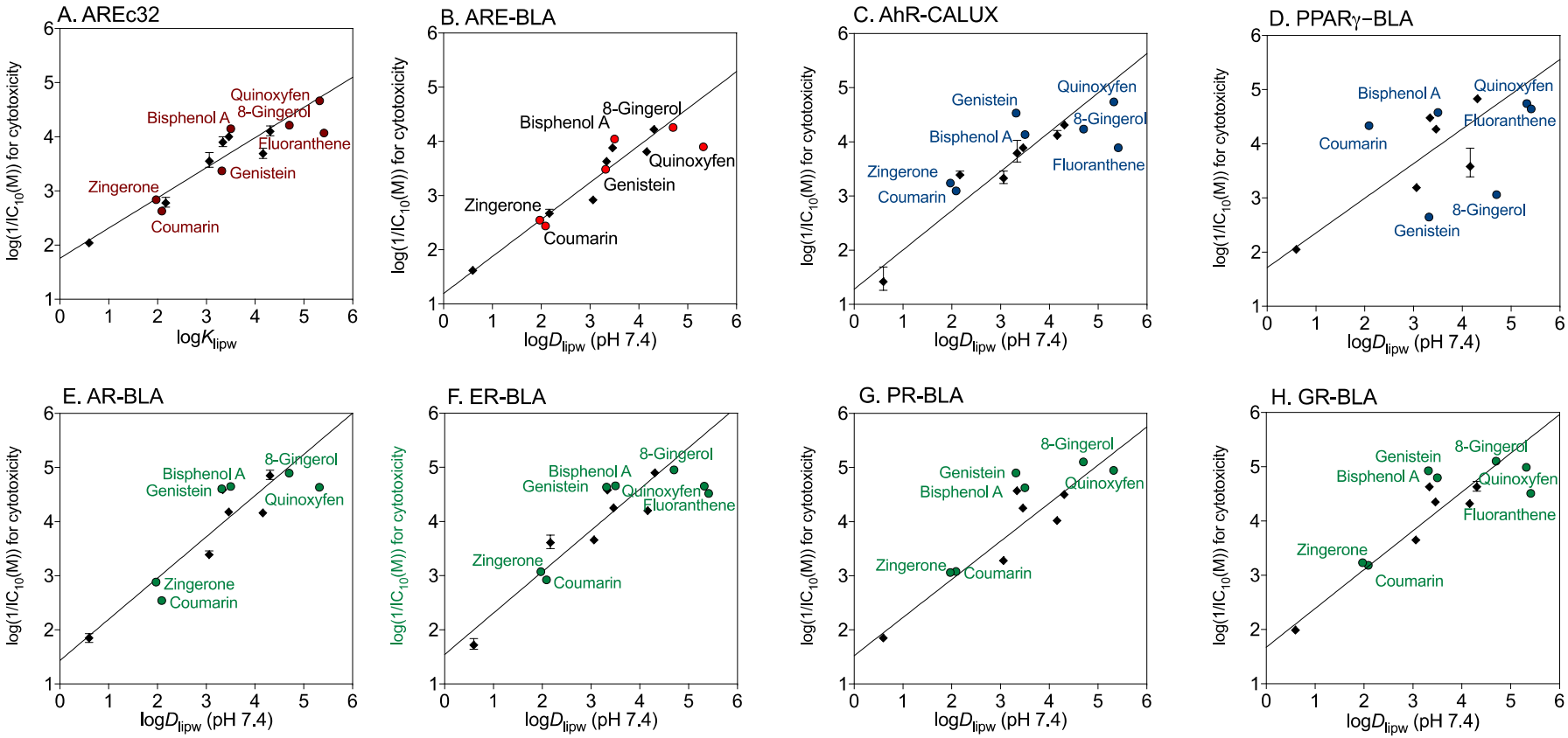


Figure S20. Comparison of the toxic ratio (TR) analysis of the entire dataset (legend "all", data from Figure 4A) in comparison to the two subsets of neutral ($f_{neutral}$ > 98%) and (partially) charged ($f_{neutral}$ < 98%) chemicals.

The underlying data are in the EXCEL Tables S1 to S6. The number of chemicals n for which a TR could be derived n(TR) is given in the top row. The percentages in the top refer to all data with TR > 10 (black diamond symbols), the percentages in the bottom refer to all data with TR ≤ 10 (red circle symbols).

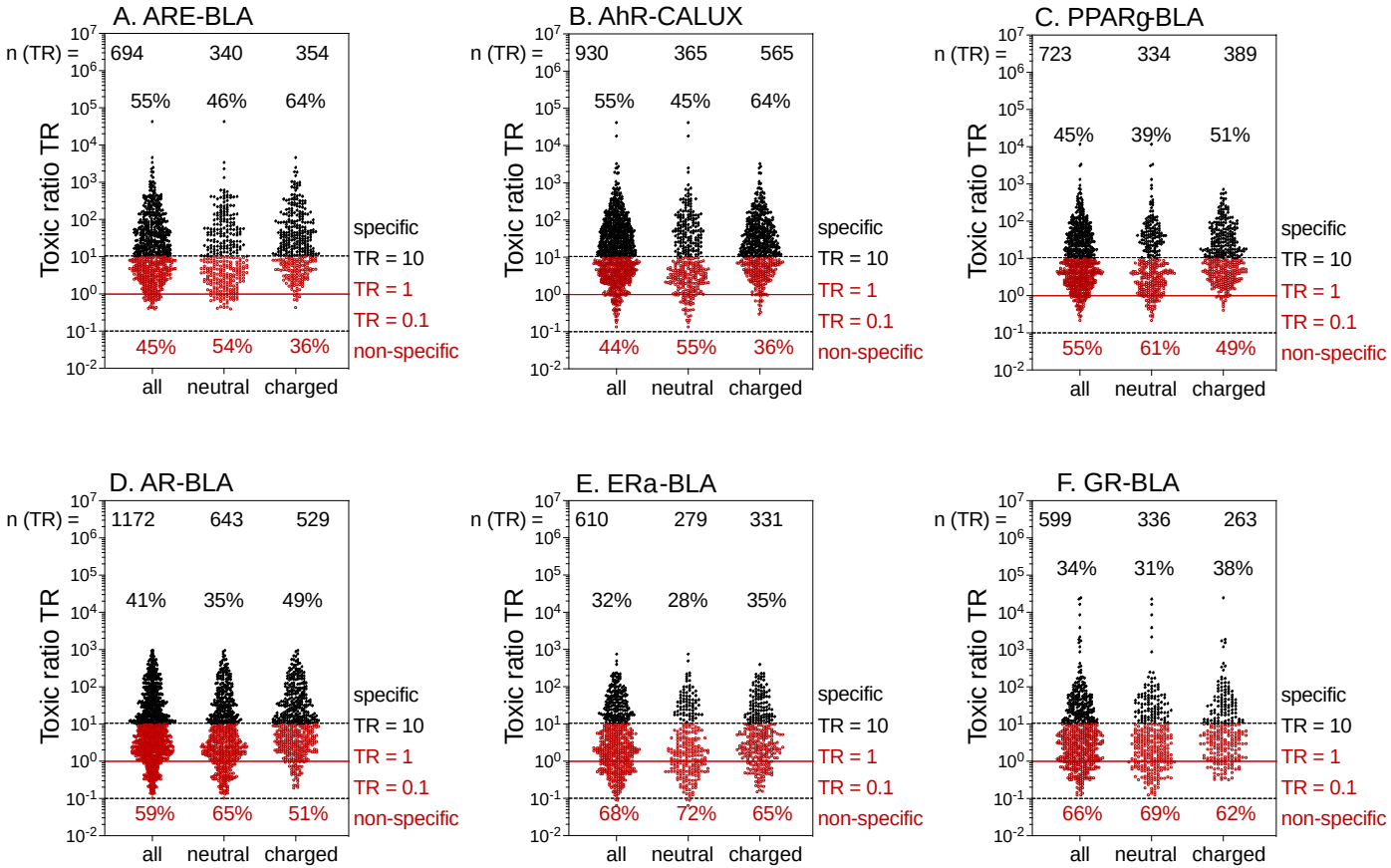


Figure S21. Comparison EC_{10} derived from the results of the individual Tox21 laboratories (black dots) compared to the joint data evaluation of all data (red diamonds) of a given chemical in this study. Selected examples are depicted for ERα-BLA. They demonstrate that the joint evaluation is reasonable.

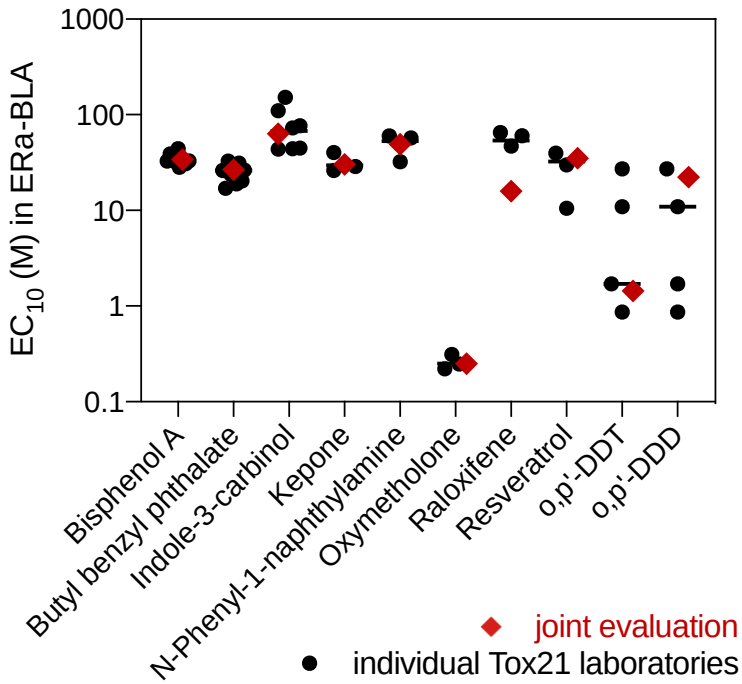


Figure S22. Comparison between the IC_{10}/EC_{10} values measured in the present study and the corresponding chemicals/assays in Tox21.

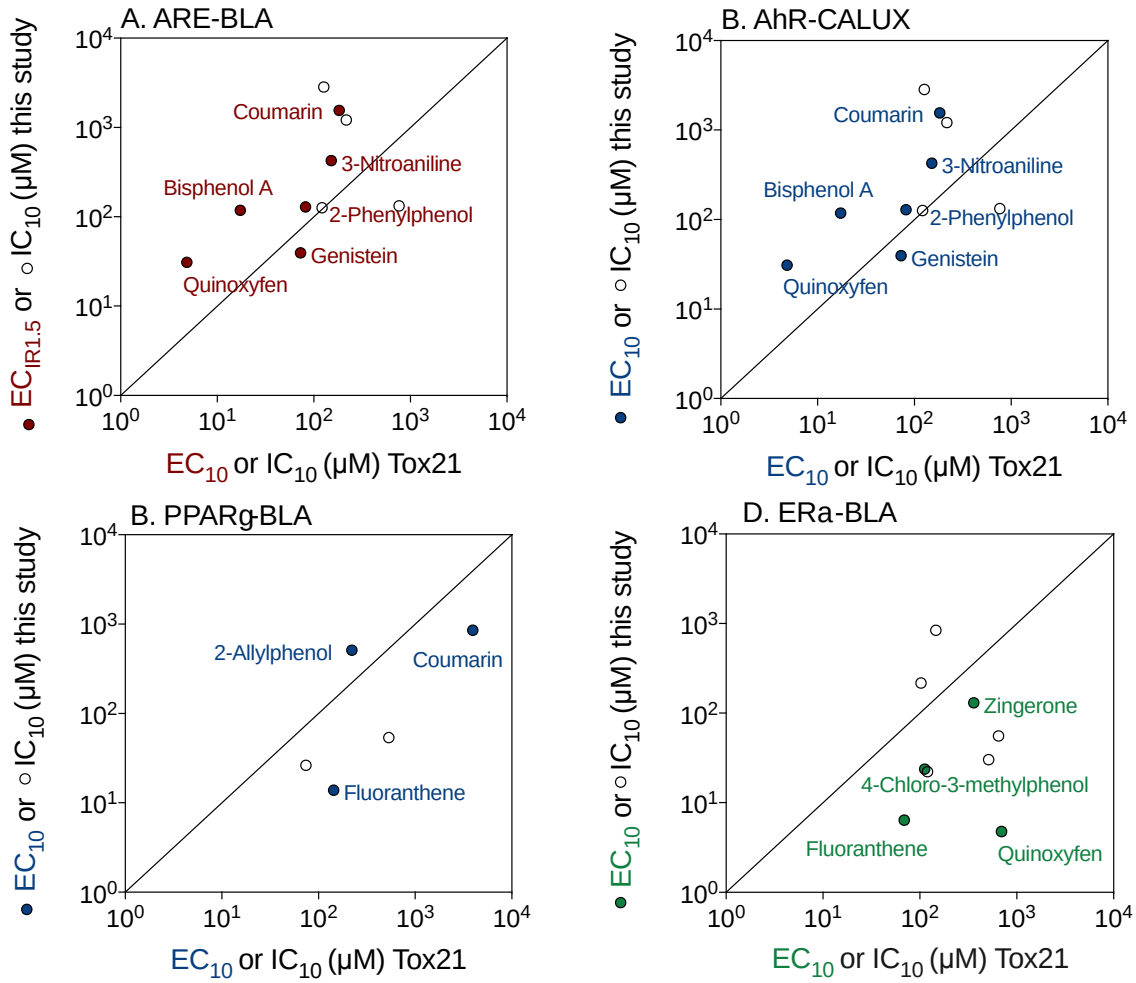
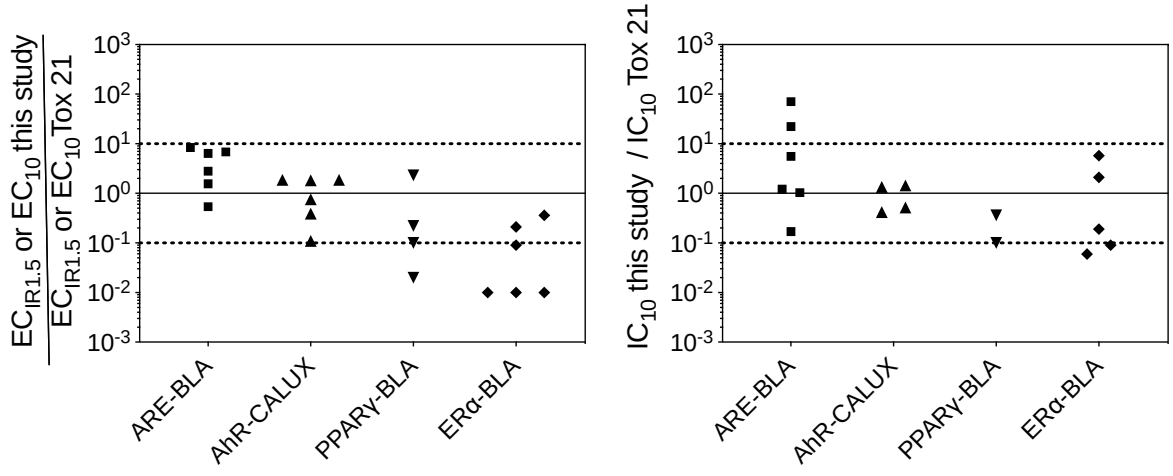


Figure S23. Ratio between the IC_{10} and EC_{10} values measured in the present study and the corresponding chemicals/assays in Tox21.



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