SUPPORTING INFORMATION

Combination of multiple neural crest function assays to identify environmental toxicants from a proof-of-concept chemical library

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1. List of antibodies used in this study

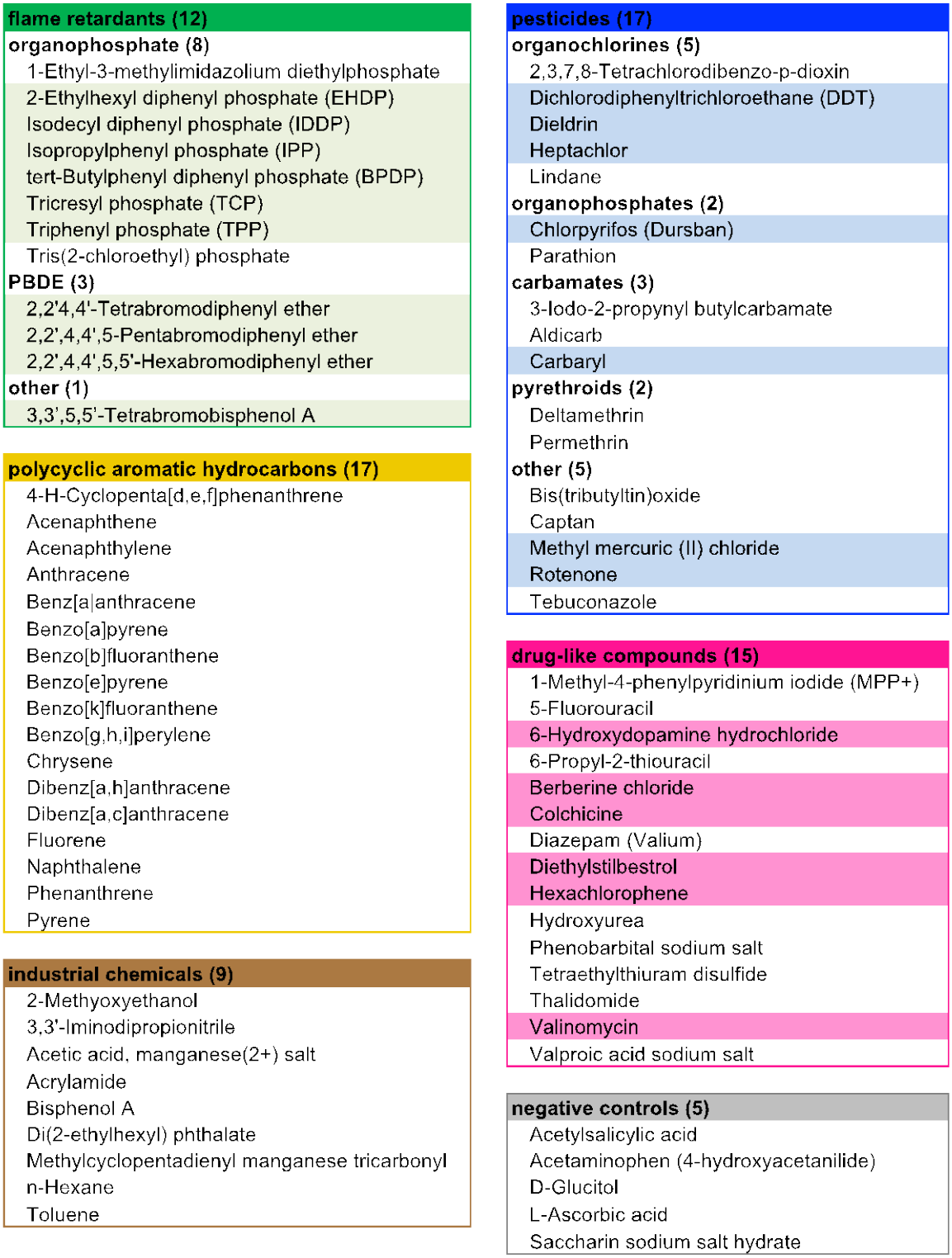
Target name	Antibody name	Host	Dilution	Catalog #	Provider
p75	Monoclonal Mouse anti-NGFR antibody (ME20.4, p75)	mouse IgG1	1:200	#AB-N07	Advanced targeting systems
Nestin	Nestin Monoclonal Antibody (clone #196908)	mouse IgG1	1:500	MAB1259	R&D Systems
α-Tubulin	Mouse monoclonal Anti-α-Tubulin antibody (clone DM1A)	mouse IgG1	1:1000	T6199	Sigma
TOM20	Tom20 Antibody (clone FL-145)	Rabbit	1:500	sc-11415	SantaCruz
Giantin	Giantin monoclonal antibody (G1/133)	mouse IgG1	1:400		Gift of H. Farhan (University of Oslo)
F-actin	Alexa Fluor® 555 Phalloidin	-	1:100	A12380	Invitrogen

2. List of toxicants used in this study

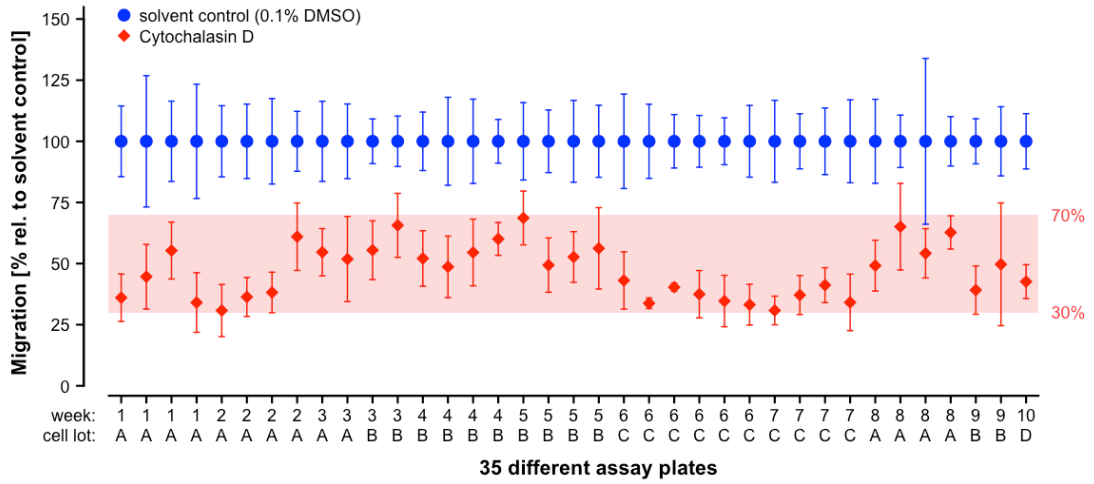
Purpose	Compound	CAS	Catalog #	Provider
endpoint-specific controls	**Cytochalasin D**	22144-77-0	C8273	Sigma
	EGCG	989-51-5	E4143	Sigma
	PP2	172889-27-9	P0042	Sigma
	Pertussis toxin	70323-44-3	P2980	Sigma
	SP600125	129-56-6	S5567	Sigma
	Taxol	33069-62-4	1097	Tocris
positive compounds	**Acrylamid**	79-06-1	A3553	Sigma
	As_2O_3	1327-53-3	11099	Sigma
	$CdCl_2$	10108-64-2	655198	Sigma
	LiCl	7447-41-8	L9650	Sigma
	Retinoic acid	302-79-4	R2625	Sigma
unspecific compounds	**L-Homocysteine thiolactone**	31828-68-9	H6503	Sigma
	MG-132	133407-82-6	S2619	Selleckchem
	Triton X-100	9002-93-1	93443	Sigma

3. List of compounds used in hit-confirmation

Compound	CAS	Stock conc.	Catalog #	Provider
confirmed compounds				
6-Hydroxydopamine	28094-15-7	100 mM	H4381-100MG	Sigma
Berberine chloride	633-65-8	20 mM	B3251	Sigma
BPDP	56803-37-3	100 mM	TRC-B693910	Biozol (TRC)
Carbaryl	63-25-2	80 mM	32055	Sigma
Chlorpyriphos	2921-88-2	100 mM	94114 Fluka	Sigma / TraceCERT
Colchicine	64-86-8	10 mM in H_2O	C9754	Sigma
DDT	50-29-3	100 mM	N11567 Supelco	Sigma / Supelco
Dieldrin	60-57-1	100 mM	33491	Sigma
Diethylstilbestrol	56-53-1	100 mM	D4628	Sigma
EHDP	1241-94-7	100 mM	34064-100MG-R	Sigma / Pestanal
Heptachlor	76-44-8	100 mM	P-053N-250	amchro
Hexachlorophene	70-30-4	100 mM	45526-250MG	Sigma / Pestanal
IDDP	29761-21-5	50 mM	PLAS-PL-022N	amchro (AccuStandard)
IPP (Kronitex 100)	68937-41-7	40 mM	NG-13725-1G	Chem Service
MeHgCl	115-09-3	4 mM in H_2O	VHG-MMC-25	LGC Standards
PBDE-47	5436-43-1	100 mM	TRC-T291145	Biozol (TRC)
PBDE-99	60348-60-9	40 mM		Clickchem
PBDE-153	68631-49-2	10 mM		obtained from NTP
Rotenone	83-79-4	100 mM	R8875	Sigma
TB-BPA	79-94-7	100 mM	11223-100MG	Sigma
Tricresyl phosphate	1330-78-5	100 mM	P-209N-250	amchro
Triphenyl phosphate	115-86-6	100 mM	241288	Sigma
Valinomycin	2001-95-8	20 mM	94675	Sigma
op'-DDT	789-02-6	100 mM	IPO 125	LGC Standards
pp'-DDT	50-29-3	100 mM	DRE-C12082000	LGC Standards
not confirmed compounds				
Acenaphthylene	208-96-8	100 mM	92549 Fluka	Sigma / TraceCERT
Bisphenol A	80-05-7	100 mM	BPA-A-N	amchro (Accustandard)
Dibenz[a,c]anthracene	215-58-7	40 mM	DRE-C20695000	LGC

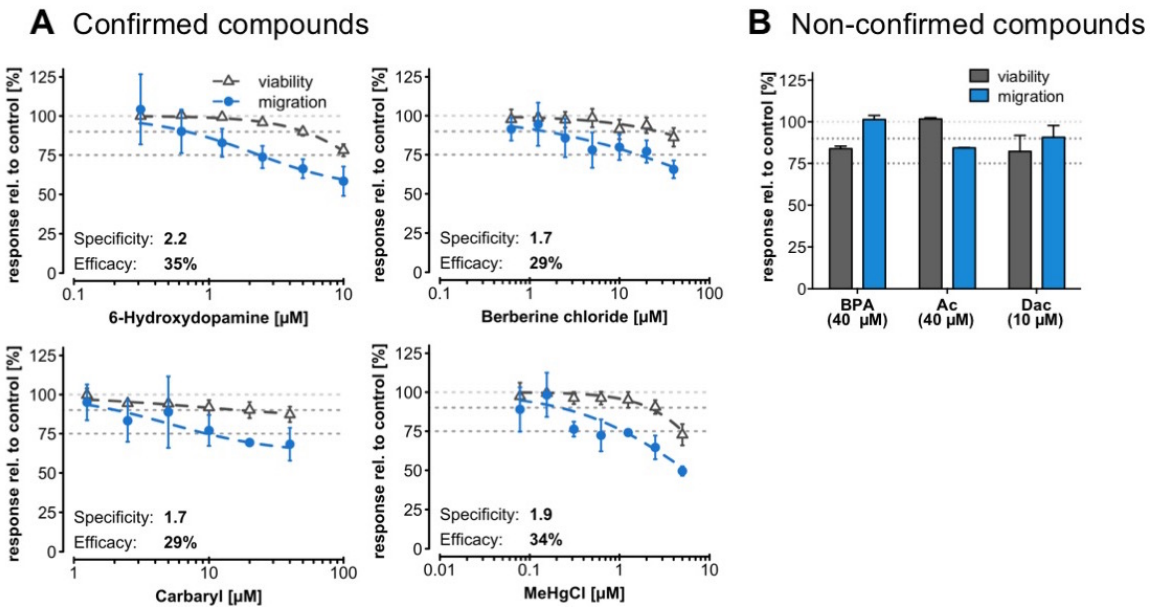


Supporting information Fig. S1. Overview of the screen library.
All library compounds were grouped into one of six chemical classes. The compounds marked with color were identified as positive hits in the hit confirmation testing. They comprise 10 of 12 flame retardants, 7 of 17 pesticides and 6 of 15 drug-like compounds whereas all polycyclic aromatic hydrocarbons, industrial chemicals and negative controls were identified as ‚negatives'.



Supporting information Fig. S2. Performance of the assay.

Cytochalasin D was run as a positive control on every assay plate. Displayed are the measured migration for cytochalasin D (in red) and the variance of the solvent control (in blue) over 35 different assay plates run in different weeks and using various cell lots.



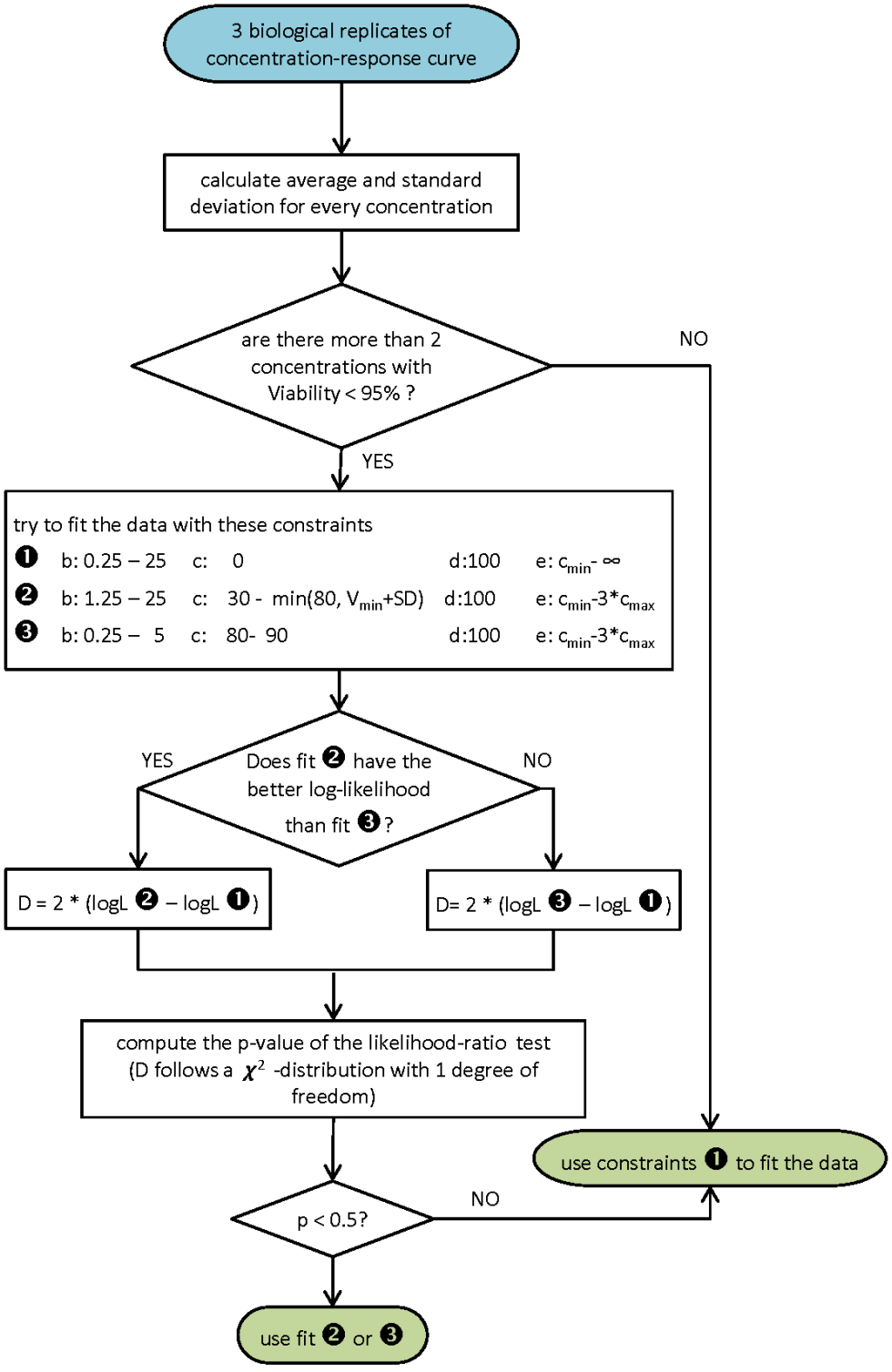
Supporting information Fig. S3. Hit confirmation with primary assay.

Compounds identified as potential hits in the screen were re-ordered and concentration-response curves were obtained for viability (gray triangles) and migration (blue circles). All values are normalized to the solvent control (0.1% DMSO). The horizontal light gray dotted line indicates the 100% value for easier reading of the diagrams. The other two gray lines are drawn at 90% and 75% to indicate the threshold for reduced viability and migration, respectively. A log-logistic function with constraints was fitted to the concentration-response curve and the EC90 of viability and the EC75 of migration were intrapolated. The ratio between these two values was termed ‚specificity', whereas ‚efficiency' was defined as the amount of migration-inhibition at the EC90 of viability.

(A) Concentration-response curves from additional confirmed hits from the group of pesticides and drug-like compounds. Data are means±SD from three experiments.

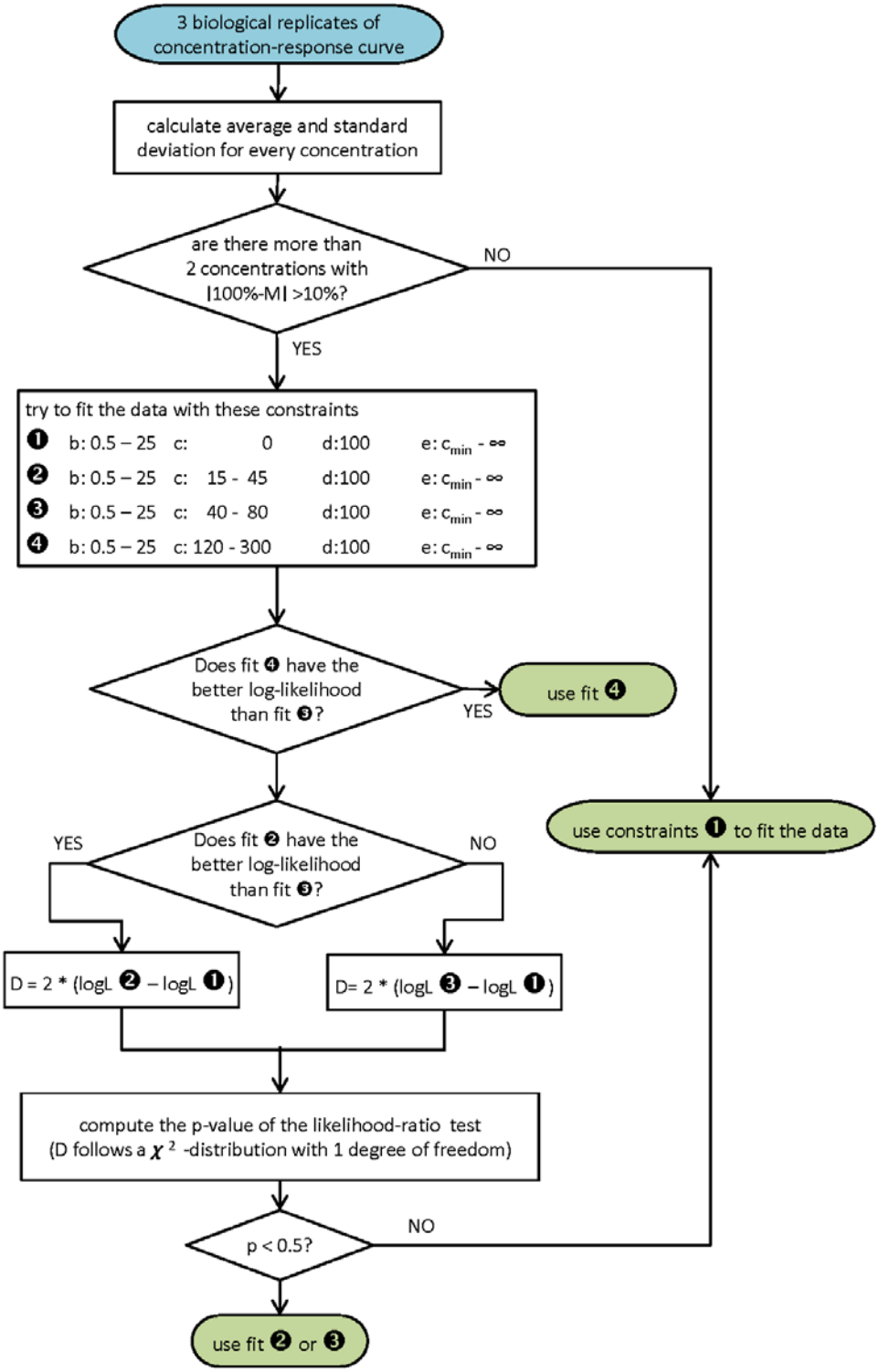
(B) Non-confirmed hits, i.e. compounds where at the highest tested concentration (displayed in brackets) no migration-inhibition occurred. Data are means±SD from two experiments.

Abbreviations: BPA: bisphenol A; Ac: acenaphthylene; Dac: dibenz[a,c]anthracene.



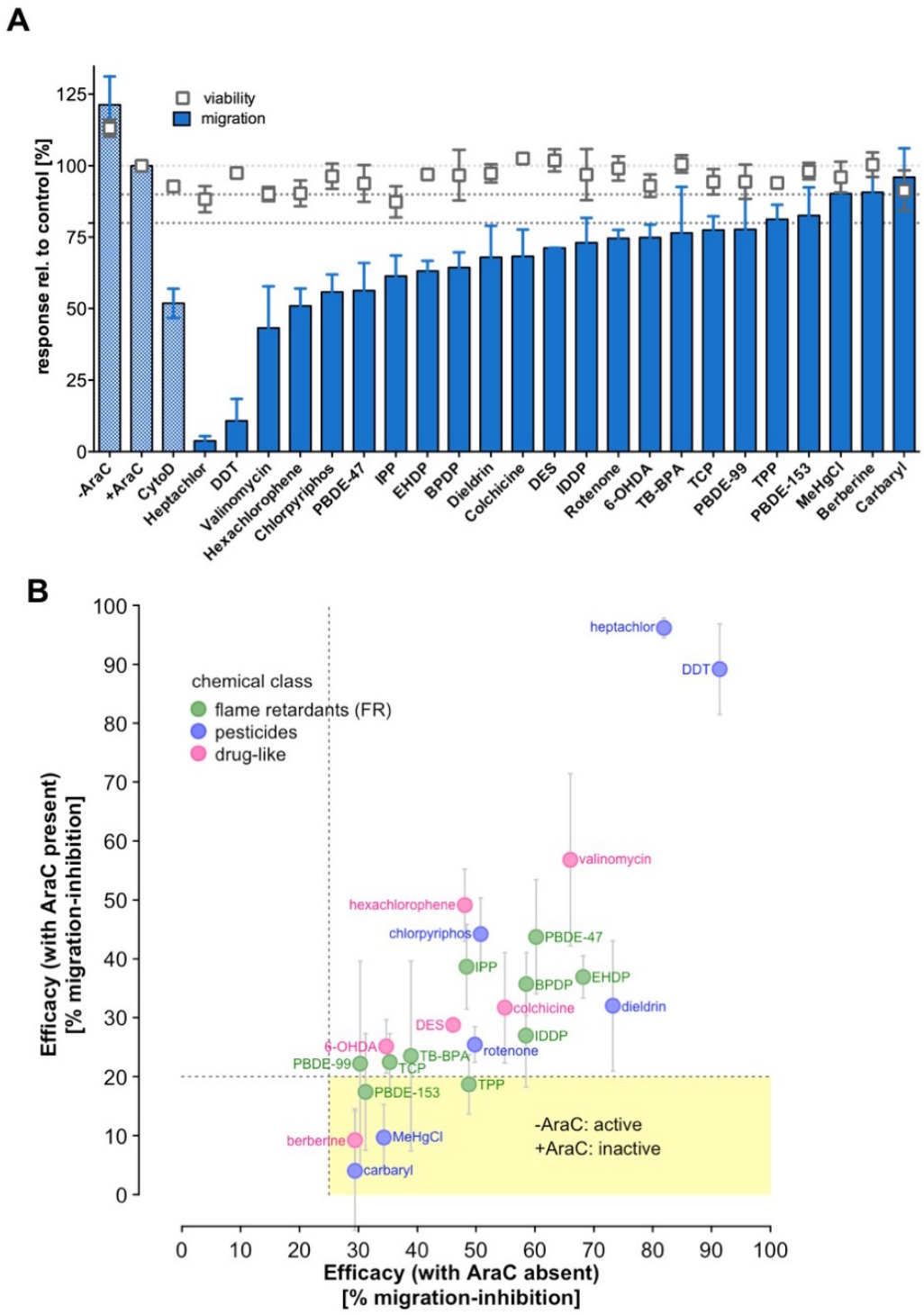
Supporting information Fig. S4. Overview of the procedure to fit concentration-response curves for viability data.

The data of three independent experiments were averaged and fitted with log-logistic functions with different constraints and fixed values. Using a likelihood-ratio test, it was tested whether it was justifiable to use a function with three degrees of freedom (curves 2 and 3) over the one with two degrees of freedom (curve 1, fixed values at 0 and 100%).



Supporting information Fig. S5. Overview of the procedure to fit concentration-response curves for migration data.

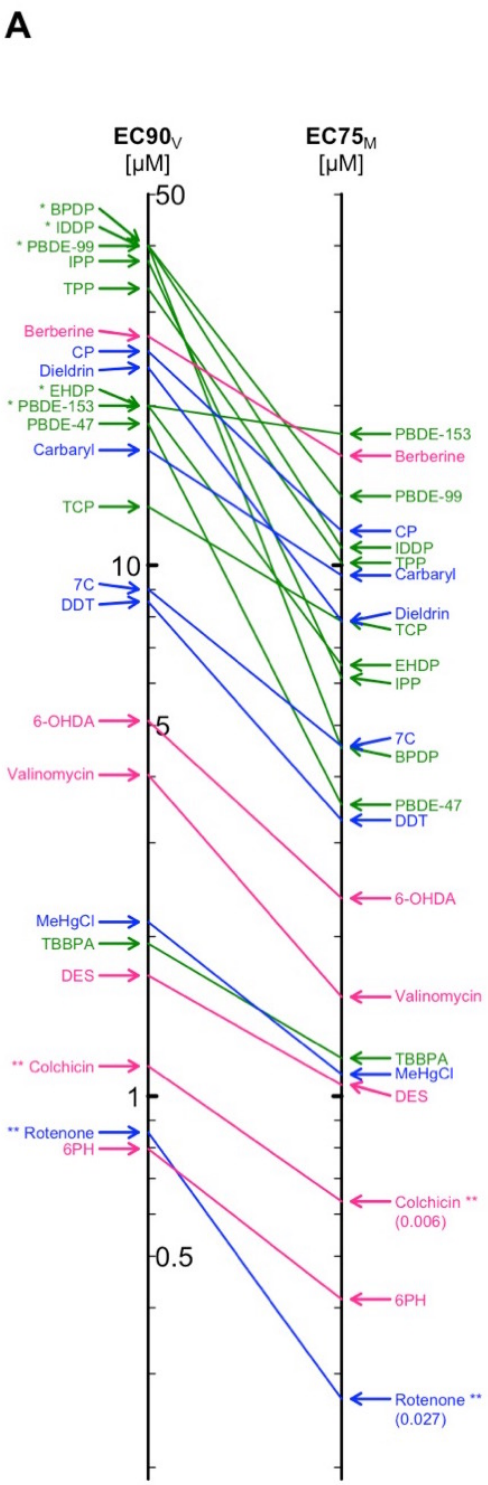
The data of three independent experiments were averaged and fitted with log-logistic functions with different constraints and fixed values. In a first step, it was tested whether the data indicate an increase in migration (curve 4). If this was not the case, a likelihood-ratio test was performed to test whether it was justifiable to use a function with three degrees of freedom (curves 2 and 3) over the one with two degrees of freedom (curve 1, fixed values at 0 and 100%).



Supporting information Fig. S6. Retesting of confirmed hits under conditions that prevent proliferation.

(A) All confirmed hits were retested in the cMINC assay under conditions that prevent proliferation (addition of 1 µM cytosine arabinoside (AraC) during the toxicant exposure). Compounds were tested at a single concentration (the $EC90_V$ or the highest non-cytotoxic concentration if the $EC90_V$ was not reached). All results were normalized to the control (1 µM AraC, 0.1% DMSO). Data are means±SD from at least two experiments. (B) Representation of the results in absence of proliferation (+ AraC) compared to the efficacy from the hit-confirmation testing (- AraC).

Abbreviations: CytoD: cytochalasin D; TB-BPA: 3,3',5,5'-tetrabromobisphenol A; 6-OHDA: 6-hydroxydopamine hydrochloride; DES: diethylstilbestrol.



B orderd by specificity

Compound	Specificity
BPDP	≥ 8.8
IPP	6.1
PBDE-47	5.2
IDDP	≥ 3.7
TPP	3.3
Rotenone	3.2
EHDP	≥ 3.1
Dieldrin	3.0
PBDE-99	≥ 3.0
Valinomycin	2.6
DDT	2.6
Chlorpyriphos	2.2
6-Hydroxydopamine	2.2
Heptachlor	2.0
MeHgCl	1.9
Hexachlorophene	1.9
Colchicin	1.8
Carbaryl	1.7
Berberine chloride	1.7
TCP	1.6
Tetrabromobisphenol A	1.6
Diethylstilbestrol	1.6
PBDE-153	≥ 1.1

C ordered by efficacy

Compound	Efficacy
DDT	91
Heptachlor	82
Dieldrin	73
EHDP	≥ 68
Valinomycin	66
PBDE-47	60
BPDP	≥ 59
IDDP	≥ 58
Colchicin	55
Chlorpyriphos	51
Rotenone	50
TPP	49
IPP	48
Hexachlorophene	48
Diethylstilbestrol	46
Tetrabromobisphenol A	39
TCP	35
6-Hydroxydopamine	35
MeHgCl	34
PBDE-153	≥ 31
PBDE-99	≥ 30
Carbaryl	29
Berberine chloride	29

Supporting information Fig. S7. Summary of the cMINC assay results.
(A) Schematic indicating the potencies of the confirmed hits displayed as EC90 of viability and EC75 of migration. Compounds with high specificity have a steep connection line. *: compounds for which the highest tested concentration was not cytotoxic; **: EC values for rotenone and colchicine were multiplied with 10 and 100, respectively, to fit on the scale. (B) List of all confirmed hit compounds ordered from high specificity (top) to low specificity (bottom). (C) List of all confirmed hit compounds ordered from high efficacy (top) to low efficacy (bottom).
The color code represents the chemical classes: green: flame retardants; blue: pesticides; pink: drug-like compounds.

	cMINC				cMINC + AraC	Cell Tracking		Transwell	
Compound	EC90V [µM]	EC75M [µM]	Specificity	Efficacy	Efficacy	conc	Efficacy	conc	Efficacy
BPDP	> 40	4.5	≥ 8.8	≥ 59%	36%		19%		58%
EHDP	> 20	6.5	≥ 3.1	≥ 68%	37%				49%
IDDP	> 40	11	≥ 3.7	≥ 58%	27%				44%
IPP	37	6.1	6.1	48%	39%				41%
TCP	13	7.8	1.6	35%	22%				30%
TPP	33	10	3.3	49%	19%				48%
PBDE-47	19	3.5	5.2	60%	44%		13%		45%
PBDE-99	> 40	14	≥ 3.0	≥ 30%	22%			*20 uM*	44%
PBDE-153	> 20	18	≥ 1.1	≥ 31%	17%				34%
TB-BPA	1.9	1.2	1.6	38%	24%		6%		37%
DDT	8.5	3.3	2.6	91%	89%		54%		39%
Dieldrin	24	7.9	3.0	46%	32%		7%		52%
Heptachlor	9.0	4.6	2.0	82%	96%		65%		40%
Chlorpyriphos	25	12	2.2	51%	44%		11%		35%
Carbaryl	17	9.6	1.7	29%	4%		22%		57%
MeHgCl	2.1	1.1	1.9	34%	10%		11%		55%
Rotenone	0.086	0.027	3.2	50%	25%		12%		46%
6-OHDA	5.1	2.4	2.2	35%	25%		15%		56%
Berberine	27	16	1.7	29%	9%	*20 uM*	12%	*20 uM*	56%
Colchicine	0.011	0.0063	1.8	55%	32%		-9%		37%
DES	1.7	1.1	1.6	73%	29%		-5%		40%
Hexachlorophene	0.80	0.42	1.9	48%	49%		32%		33%
Valinomycin	4.0	1.5	2.6	66%	57%	*1.25 uM*	-9%	*1.25 uM*	47%

Supporting information Table S1. Summary of the results from all assays.
Quantitative results from all four migration assays used in this study. For the standard assay (cMINC), the EC90V (EC90 of viability) and EC75M (EC75 of migration) were obtained by curve fitting of the concentration-response data. If the fitted value was above the highest tested concentration, the '>' sign was introduced. 'Specificity' was defined as the ratio of EC90V and EC75M, whereas 'efficacy' was defined as the migration-inhibition at the fitted EC90V. For all other assays, compounds were tested at the EC90V of the cMINC assay or at the indicated concentration. The efficacy refers to the migration-inhibition of this test concentration.