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Table S1: Scientific Advisory Committee

Member	Affiliation	Area of Expertise
Bruce Blumberg, PhD	University of California, Irvine	Endocrine Disruption
Terrence Collins, PhD	Carnegie Mellon University	Green Chemistry
David Crews, PhD	University of Texas at Austin	Endocrine Disruption
Peter L. deFur, PhD	Environmental Stewardship Concepts, LLC	Endocrine disruption
Andrea C. Gore, PhD	University of Texas at Austin	Endocrine Disruption
Lou Guillette, PhD	Medical University of South Carolina	Endocrine Disruption
Jerrold Heindel, PhD	National Institute of Environmental Health Sciences	Endocrine disruption
John Peterson Myers, PhD	Environmental Health Sciences	Endocrine disruption
Kristina A. Thayer, PhD	Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program	Endocrine Disruption
Frederick S. vom Saal, PhD	University of Missouri	Endocrine Disruption
John Warner, PhD	Warner Babcock Institute for Green Chemistry	Green Chemistry
Cheryl S. Watson, PhD	University of Texas Medical Branch	Endocrine Disruption
R. Thomas Zoeller, PhD	University of Massachusetts, Amherst	Endocrine Disruption

Table S2: Tools available for in-house computational-based assessments of EDC

activity

Database	Website	Summary
FDA Endocrine Disruptor Knowledge Base (EDKB)	http://edkb.fda.gov/webstart/edkb/	Approximately 3300 records for over 1800 EDCs from different assays. Data can be cross-linked to other publicly available databases including TOXNET
MOLE db	http://michem.distat.unimib.it/mole_db/	Molecular Descriptors Database by Milano Chemometric and QSAR Research Group. The MOLE bd is a free online database of molecular descriptors calculated for 243773 molecules.
TOXNET	http://toxnet.nlm.nih.gov/	Databases on toxicology, hazardous chemicals, environmental health, and toxic releases.
VEGA	http://www.vega-qsar.eu/	Free platform for QSAR modeling
CAESAR (Computer Assisted Evaluation of industrial chemical Substances According to Regulations)	http://www.caesar-project.eu/	 CAESAR was formed to develop QSAR models for REACH legislation. Five endpoints: bioconcentration factor skin sensitization carcinogenicity mutagenicity developmental toxicity
VirtualToxLab	http://www.biograf.ch/	Tool for predicting the toxic potential (endocrine and metabolic disruption) of drugs, chemicals and natural products. It simulates and quantifies their interactions towards a series of proteins known to trigger adverse effects using automated, flexible docking combined with multi-dimensional QSAR (mQSAR).
3D QSAR	www.3d-qsar.com	3D QSAR Models Database
Open3DQSAR	www.open3dqsar.org	Open-source tool aimed at pharmacophore exploration by high-throughput chemometric analysis of

		molecular interaction fields (MIFs).
EPA ACTor	http://actor.epa.gov/actor/	ACToR aggregates data from over 500 public sources on over 500,000 environmental chemicals searchable by chemical name, other identifiers and by chemical structure.
NTP CEBS	http://www.niehs.nih.gov/research/resources/d	The CEBS database
	atabases/cebs/index.cfm	houses data on chemical effects on biological systems that have been deposited by academic, industrial and governmental laboratories.
PubChem GeneGO	http://www.genego.com/	Data mining tools and databases help to capture and define the underlying biology behind different types of high-throughput experimental data and understand the effects of small molecule drug compounds in human tissues.
Comparative	http://ctdbase.org/	CTD includes curated data
Toxicogenomics Database		describing cross-species chemical–gene/protein interactions and chemical– and gene–disease associations to illuminate molecular mechanisms underlying variable susceptibility and environmentally influenced diseases.
Leadscope	http://www.leadscope.com/	Incorporates chemically based data mining, visualization and advanced informatics techniques.
OECD QSAR Toolbox	http://www.oecd.org/document/54/0,3746,en_	A software application
	2649_34379_42923638_1_1_1_1,00.html	designed to fill in gaps in (eco)toxicity data needed for hazard assessment of chemicals.
OpenTox	http://www.opentox.org/	Tools for the integration of data from various sources to generate and validate computer models for toxic effects

Table S3: Receptors and other endpoints that can be assessed using Tier 2 high-throughput screening

Androgen receptorAryl hydrocarbon receptorEstrogen receptor alphaEstrogen receptor betaFarnesoid X receptorGlucagon receptorGlucocorticoid receptor (GR)Liver X receptor β (LXR β)Melanin-concentrating hormone receptor 1 (MCHR1)Membrane estrogen receptor (mER/GPR30)Mineralocorticoid receptorPeroxisome proliferator-activated receptor a, δ, γ (PPAR a, PPARō, PPARγ)Pregnane X receptorProlactin receptor (PRLR)Prostaglandin agonism through EP1 receptorRetinoic acid receptor α (RXRα)Retinoid X receptor α (RXRα)Retinoid X receptor b (TRb)Vasopressin V3 (V1B) ReceptorVitamin D receptor (VDR)	
Estrogen receptor alphaEstrogen receptor betaFarnesoid X receptorGlucagon receptorGlucocorticoid receptor (GR)Liver X receptor β (LXR β)Melanin-concentrating hormone receptor 1 (MCHR1)Membrane estrogen receptor (mER/GPR30)Mineralocorticoid receptorPeroxisome proliferator-activated receptor a, δ, γ (PPAR a, PPAR δ , PPAR γ)Pregnane X receptorProlactin receptor (PRLR)Prostaglandin agonism through EP1 receptorRetinoic acid receptor α (RXR α)Retinoid X receptor α (RXR α)Retinoid-related orphan receptor gamma (ROR γ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	Androgen receptor
Estrogen receptor betaFarnesoid X receptorGlucagon receptorGlucocorticoid receptor (GR)Liver X receptor β (LXR β)Melanin-concentrating hormone receptor 1 (MCHR1)Membrane estrogen receptor (mER/GPR30)Mineralocorticoid receptorPeroxisome proliferator-activated receptor a, δ, γ (PPAR a, PPARδ, PPARγ)Pregnane X receptorProlactin receptor (PRLR)Prostaglandin agonism through EP1 receptorRetinoic acid receptor α (RXR α)Retinoid X receptor α (RXR α)Retinoid-related orphan receptor gamma (RORγ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	Aryl hydrocarbon receptor
Farnesoid X receptorGlucagon receptorGlucocorticoid receptor (GR)Liver X receptor β (LXRβ)Melanin-concentrating hormone receptor 1 (MCHR1)Membrane estrogen receptor (mER/GPR30)Mineralocorticoid receptorPeroxisome proliferator-activated receptor a, δ , γ (PPAR a, PPAR δ , PPAR γ)Pregnane X receptorProlactin receptor (PRLR)Prostaglandin agonism through EP1 receptorRetinoic acid receptor α (RXR α)Retinoid X receptor α (RXR α)Retinoid-related orphan receptor gamma (ROR γ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	Estrogen receptor alpha
Glucagon receptorGlucocorticoid receptor (GR)Liver X receptor β (LXRβ)Melanin-concentrating hormone receptor 1 (MCHR1)Membrane estrogen receptor (mER/GPR30)Mineralocorticoid receptorPeroxisome proliferator-activated receptor a, δ , γ (PPAR a, PPAR δ , PPAR γ)Pregnane X receptorProlactin receptor (PRLR)Prostaglandin agonism through EP1 receptorRetinoic acid receptor α (RXR α)Retinoid X receptor α (RXR α)Retinoid-related orphan receptor gamma (ROR γ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	Estrogen receptor beta
Glucocorticoid receptor (GR)Liver X receptor β (LXR β)Melanin-concentrating hormone receptor 1 (MCHR1)Membrane estrogen receptor (mER/GPR30)Mineralocorticoid receptorPeroxisome proliferator-activated receptor a, δ, γ (PPAR a, PPARδ, PPARγ)Pregnane X receptorProlactin receptor (PRLR)Prostaglandin agonism through EP1 receptorRetinoic acid receptor (RAR)Retinoid X receptor α (RXRα)Retinoid-related orphan receptor gamma (RORγ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	Farnesoid X receptor
Liver X receptor β (LXR β) Melanin-concentrating hormone receptor 1 (MCHR1) Membrane estrogen receptor (mER/GPR30) Mineralocorticoid receptor Peroxisome proliferator-activated receptor a, δ , γ (PPAR a, PPAR δ , PPAR γ) Pregnane X receptor Prolactin receptor (PRLR) Prostaglandin agonism through EP1 receptor Retinoic acid receptor (RAR) Retinoid X receptor α (RXR α) Retinoid-related orphan receptor gamma (ROR γ) Thyroid hormone receptor b (TRb) Vasopressin V3 (V1B) Receptor	Glucagon receptor
Melanin-concentrating hormone receptor 1 (MCHR1)Membrane estrogen receptor (mER/GPR30)Mineralocorticoid receptorPeroxisome proliferator-activated receptor a, δ, γ (PPAR a, PPARδ, PPARγ)Pregnane X receptorProlactin receptor (PRLR)Prostaglandin agonism through EP1 receptorRetinoic acid receptor (RAR)Retinoid X receptor α (RXRα)Retinoid-related orphan receptor gamma (RORγ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	Glucocorticoid receptor (GR)
(MCHR1)Membrane estrogen receptor (mER/GPR30)Mineralocorticoid receptorPeroxisome proliferator-activated receptor a, δ , γ (PPAR a, PPAR δ , PPAR γ)Pregnane X receptorProlactin receptor (PRLR)Prostaglandin agonism through EP1 receptorRetinoic acid receptor (RAR)Retinoid X receptor α (RXR α)Retinoid-related orphan receptor gamma (ROR γ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	Liver X receptor β (LXR β)
Membrane estrogen receptor (mER/GPR30)Mineralocorticoid receptorPeroxisome proliferator-activated receptor a, δ, γ(PPAR a, PPARō, PPARǫ)Pregnane X receptorProlactin receptor (PRLR)Prostaglandin agonism through EP1 receptorRetinoic acid receptor (RAR)Retinoid X receptor α (RXR α)Retinoid-related orphan receptor gamma (RORǫ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	Melanin-concentrating hormone receptor 1
$\begin{array}{l} \mbox{Mineralocorticoid receptor} \\ \mbox{Peroxisome proliferator-activated receptor a, δ, γ} \\ \mbox{(PPAR a, PPARδ, PPAR\geta)} \\ \mbox{Pregnane X receptor} \\ \mbox{Prolactin receptor (PRLR)} \\ \mbox{Prostaglandin agonism through EP1 receptor} \\ \mbox{Retinoic acid receptor (RAR)} \\ \mbox{Retinoid X receptor α (RXRα)} \\ \mbox{Retinoid-related orphan receptor gamma (ROR\geta)} \\ \mbox{Thyroid hormone receptor b (TRb)} \\ \mbox{Vasopressin V3 (V1B) Receptor} \end{array}$	(MCHR1)
Peroxisome proliferator-activated receptor a, δ , γ (PPAR a, PPAR δ , PPAR γ) Pregnane X receptor Prolactin receptor (PRLR) Prostaglandin agonism through EP1 receptor Retinoic acid receptor (RAR) Retinoid X receptor α (RXR α) Retinoid-related orphan receptor gamma (ROR γ) Thyroid hormone receptor b (TRb) Vasopressin V3 (V1B) Receptor	Membrane estrogen receptor (mER/GPR30)
$\begin{array}{l} (PPAR a, PPAR\delta, PPAR\gamma) \\ \hline Pregnane X receptor \\ \hline Prolactin receptor (PRLR) \\ \hline Prostaglandin agonism through EP1 receptor \\ \hline Retinoic acid receptor (RAR) \\ \hline Retinoid X receptor \alpha (RXR\alpha) \\ \hline Retinoid-related orphan receptor gamma (ROR\gamma) \\ \hline Thyroid hormone receptor b (TRb) \\ \hline Vasopressin V3 (V1B) Receptor \\ \end{array}$	Mineralocorticoid receptor
Pregnane X receptorProlactin receptor (PRLR)Prostaglandin agonism through EP1 receptorRetinoic acid receptor (RAR)Retinoid X receptor α (RXRα)Retinoid-related orphan receptor gamma (RORγ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	Peroxisome proliferator-activated receptor a, δ , γ
Prolactin receptor (PRLR)Prostaglandin agonism through EP1 receptorRetinoic acid receptor (RAR)Retinoid X receptor α (RXRα)Retinoid-related orphan receptor gamma (RORγ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	(PPAR a, PPARδ , PPARγ)
Prostaglandin agonism through EP1 receptorRetinoic acid receptor (RAR)Retinoid X receptor α (RXRα)Retinoid-related orphan receptor gamma (RORγ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	Pregnane X receptor
Retinoic acid receptor (RAR)Retinoid X receptor α (RXRα)Retinoid-related orphan receptor gamma (RORγ)Thyroid hormone receptor b (TRb)Vasopressin V3 (V1B) Receptor	Prolactin receptor (PRLR)
Retinoid X receptor α (RXRα) Retinoid-related orphan receptor gamma (RORγ) Thyroid hormone receptor b (TRb) Vasopressin V3 (V1B) Receptor	Prostaglandin agonism through EP1 receptor
Retinoid-related orphan receptor gamma (RORγ) Thyroid hormone receptor b (TRb) Vasopressin V3 (V1B) Receptor	Retinoic acid receptor (RAR)
Thyroid hormone receptor b (TRb) Vasopressin V3 (V1B) Receptor	Retinoid X receptor a (RXRa)
Vasopressin V3 (V1B) Receptor	Retinoid-related orphan receptor gamma (RORy)
	Thyroid hormone receptor b (TRb)
Vitamin D receptor (VDR)	Vasopressin V3 (V1B) Receptor
	Vitamin D receptor (VDR)

Table S4: Examples of current assays, biological endpoints, and references, available for Tier 3 screening

	Hypoth	alamic-Pituitary-Adrenal	(Stress) Axis	
Assay	Receptor	Cell Type	Endpoints	Reference(s)
Glucocorticoid responsive assay	GR	Human breast cancer cells (MDA- MB-453) stably transfected with an MMTV.luciferase.neo reporter gene construct	Activation of MMTV luciferase reporter occurs via treatment with glucocorticoids or androgen receptor agonists. Treatment with anti-androgens allows GR- agonists to be examined separately	[59]
	Hypothalar	nic-Pituitary-Gonadal (Re	eproductive) Axis	
Assay	Receptor	Cell Type	Endpoints	Reference(s)
A-screen	AR	MCF-7 cells transfected with androgen receptor (MCF7-AR1)	Estrogen-induced cell proliferation is inhibited by androgens	[60]
AR-CALUX	AR	U2-OS human osteosarcoma transfected with luciferase reporter	Androgen receptor- mediated luciferase reporter gene- expression	[61]
Aromatase induction		Human adrenocortical carcinoma (H295R) Human placental choriocarcinoma (JEG-3) Human breast cancer (MCF-7)	Aromatase activity as measured by conversion of androstenedione and induction of aromatase gene expression	[62]
E-screen	ER	Human breast cancer cell line MCF-7	Cell proliferation	[63-68]
E2SULT		Cell-free	Inhibition of estrogen sulfotransferase	[69]
ER-CALUX	ER	T47D.Luc- human breast cancer cells transfected with luciferase reporter	Estrogen receptor- mediated luciferase reporter gene- expression	[70]
EstrArray	ER	Human breast cancer cell line MCF-7	Gene expression of estrogen-dependent genes	[71]
PR – transactivation /transcription assay	PR	HEK 293T transfected with luciferase reporter	PR-mediated luciferase reporter protein expression (luminescence)	[72]
PR-CALUX	PR	U2-OS human osteosarcoma	progesterone receptor-mediated	[73]

		transfacted with	luciforaça reportar	
		transfected with	luciferase reporter	
01 11 1		luciferase reporter	gene-expression	F7 43
Steroidogenesis		Human	Interference with	[74]
		adenocarcinoma cell	steroidogenesis-	
		line (H295R)	production of P4, T,	
			and E2	
YES, YAS, YPS,	ERα, ERβ, AR,	Yeast -	Hormone-mediated	[75, 76]
etc.	PR, GR, MR,	Saccharomyces	β-galactosidase	
	AhR.	cerevisiae	reporter gene-	
			expression	
	Hypo	othalamic-Pituitary-Thy		
Assay	Receptor	Cell Type	Endpoints	Reference(s)
Dendrite	TR	Primary Purkinje cells	TH-dependent	[77-79]
arborization			dendrite arborization	
arbonzation			of cerebellar	
La d'al a la batala a	NIIO	NIIO transferate d OLIO	Purkinje cells	[00] [04]
lodide Uptake	NIS	NIS-transfected CHO	Inhibition of iodide	[80]; [81]
		or FRTL-5 cells	uptake	
Neurite extension	TR	Rat granule cells	Granule cell neurite	[82]
		primary culture	extension	
T-screen aka	TR	Rat pituitary tumor cell	Cell proliferation	[83], [84]
GH3 Cell Assay		line GH3		
TH-reporter	TR	GH3 pituitary cells	Thyroid hormone	[85]
assay		transfected with TRE-	receptor-mediated	r - 1
		luciferase	luciferase reporter-	
		luciterase	gene expression	
TPO Inhibition	Thyroid	Cell-free	Inhibition of	[86], [87]
	Peroxidase	Cell-liee	thyroperoxidase	[00], [07]
Potin		Proliferator-Activated R		othway
Assay	Receptor	Cell Type	Endpoints	Reference(s)
Adipocyte		Mouse fibroblasts	Differentiation into	[88-91]
				100-91
differentiation	RXR/PPARg		adinaautaa	
differentiation	KXK/PPARg	(preadipocyte cell lines	adipocytes,	
differentiation assay	RXR/PPARg		accumulation of lipid	
assay		(preadipocyte cell lines 3T3-L1 or C3H10T1/2)	accumulation of lipid droplets	
	AhR	(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2	accumulation of lipid droplets Arylhydrocarbon	[92]
assay		(preadipocyte cell lines 3T3-L1 or C3H10T1/2)	accumulation of lipid droplets Arylhydrocarbon receptor-mediated	[92]
assay		(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2	accumulation of lipid droplets Arylhydrocarbon	[92]
assay		(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2	accumulation of lipid droplets Arylhydrocarbon receptor-mediated	[92]
assay		(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma	accumulation of lipid droplets Arylhydrocarbon receptor-mediated luciferase reporter	[92]
assay		(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma	accumulation of lipid droplets Arylhydrocarbon receptor-mediated luciferase reporter	[92]
assay		(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse	accumulation of lipid droplets Arylhydrocarbon receptor-mediated luciferase reporter	[92]
assay		(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7	accumulation of lipid droplets Arylhydrocarbon receptor-mediated luciferase reporter	[92]
assay		(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma	accumulation of lipid droplets Arylhydrocarbon receptor-mediated luciferase reporter	[92]
assay		(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse	accumulation of lipid droplets Arylhydrocarbon receptor-mediated luciferase reporter	[92]
assay AhR activation	AhR	(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma	accumulation of lipid droplets Arylhydrocarbon receptor-mediated luciferase reporter gene-expression	
assay		(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma Rat hepatoma cell line	accumulation of lipid droplets Arylhydrocarbon receptor-mediated luciferase reporter gene-expression	[92]
assay AhR activation	AhR	(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma Rat hepatoma cell line (H4IIE) transfected	Arylhydrocarbon gene-expression	
assay AhR activation	AhR	(preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma Rat hepatoma cell line	Arylhydrocarbon receptor-mediated luciferase reporter gene-expression Arylhydrocarbon receptor-mediated luciferase reporter	
assay AhR activation DR-CALUX	AhR	 (preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma Rat hepatoma cell line (H4IIE) transfected with luciferase reporter 	Arylhydrocarbon receptor-mediated luciferase reporter gene-expression Arylhydrocarbon receptor-mediated luciferase reporter gene-expression	[93]
assay AhR activation DR-CALUX PPAR	AhR	 (preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma Rat hepatoma cell line (H4IIE) transfected with luciferase reporter Several cell lines are 	Arylhydrocarbon receptor-mediated luciferase reporter gene-expression Arylhydrocarbon receptor-mediated luciferase reporter gene-expression PPAR-mediated	
AhR activation AhR activation DR-CALUX PPAR Transactivation	AhR	 (preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma Rat hepatoma cell line (H4IIE) transfected with luciferase reporter Several cell lines are used for this 	Arylhydrocarbon receptor-mediated luciferase reporter gene-expression Arylhydrocarbon receptor-mediated luciferase reporter gene-expression PPAR-mediated luciferase reporter	[93]
AhR activation DR-CALUX PPAR	AhR	 (preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma Rat hepatoma cell line (H4IIE) transfected with luciferase reporter Several cell lines are 	Arylhydrocarbon receptor-mediated luciferase reporter gene-expression Arylhydrocarbon receptor-mediated luciferase reporter gene-expression PPAR-mediated	[93]
assay AhR activation DR-CALUX PPAR Transactivation	AhR	 (preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma Rat hepatoma cell line (H4IIE) transfected with luciferase reporter Several cell lines are used for this 	Arylhydrocarbon receptor-mediated luciferase reporter gene-expression Arylhydrocarbon receptor-mediated luciferase reporter gene-expression PPAR-mediated luciferase reporter	[93]
assay AhR activation DR-CALUX PPAR Transactivation Reporter Assay	AhR	 (preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma Rat hepatoma cell line (H4IIE) transfected with luciferase reporter Several cell lines are used for this commercially available assay 	Arylhydrocarbon receptor-mediated luciferase reporter gene-expression Arylhydrocarbon receptor-mediated luciferase reporter gene-expression PPAR-mediated luciferase reporter	[93]
assay AhR activation DR-CALUX PPAR Transactivation Reporter Assay Pregnane X	AhR AhR PPAR	 (preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma Rat hepatoma cell line (H4IIE) transfected with luciferase reporter Several cell lines are used for this commercially available assay Human hepatoma cell 	Arylhydrocarbon receptor-mediated luciferase reporter gene-expression Arylhydrocarbon receptor-mediated luciferase reporter gene-expression PPAR-mediated luciferase reporter gene expression	[93]
AhR activation AhR activation DR-CALUX PPAR Transactivation Reporter Assay	AhR AhR PPAR	 (preadipocyte cell lines 3T3-L1 or C3H10T1/2) Human HepG2 hepatoma Human MCF7 Mouse H1L1.1c2 hepatoma Mouse MLEL1.1c1 hepatoma Rat hepatoma cell line (H4IIE) transfected with luciferase reporter Several cell lines are used for this commercially available assay 	Arylhydrocarbon receptor-mediated luciferase reporter gene-expression Arylhydrocarbon receptor-mediated luciferase reporter gene-expression PPAR-mediated luciferase reporter gene expression	[93]

Reporter Assay			reporter gene	
RAR	RAR	COS-7 cells	RAR-mediated	[96]
Transactivation			luciferase reporter	
Reporter Assay			activity	
			(luminescence)	
RXR	RXR	HEK 293T transfected	RXR-mediated	[97, 98]
Transactivation		with luciferase reporter	luciferase reporter	
Reporter Assay			gene expression	
	Non-ge	nomic Actions of Stere	oid Mimetics	
Assay	Receptor	Cell Type	Endpoints	Reference(s)
ERK activation	mER	Pituitary cell line	Phosphorylation of	[99-101]
(or other MAPKs)		(GH3/B6/F10)	ERK (or JNK or p38	
			kinases) – 96-well	
			plate immunoassay	
ERK activation	mER	Breast cancer (MCF-7)	Phosphorylation of	[102]
(or other MAPKs)			ERK (or JNK or p38	
			kinases) – 96-well	
			plate immunoassay	
ERK activation	mPR	Human breast cancer	Phosphorylation of	[103]
(or other MAPKs)		cells MDA-MB-231	ERK- detected by	
			Western Blot	
Gai activation	mER	Pituitary cell line	GTP-bound	Watson, submitted
		(GH3/B6/F10)	(activated) Gαi	
			protein– 96-well	
			plate immunoassay	
G protein	mPR	Human breast cancer	GTP-bound	[104, 105]
activation		cells MDA-MB-231	(activated) protein,	
			cAMP levels	

Table S5: Whole fish and amphibian assays

Assay	Fish species	Endpoints	Reference(s)
Corticosteroid secretion	Oncorhynchus mykiss	Corticosteroid secretion in response to ACTH	[106]
Rapid developmental toxicity HTS (in Tier 2)	Zebrafish	Morphological endpoints (edema, bent body axes, pigmentation anomalies, and organ malformations)	[40, 107-111]
Fish sex development test	Fathead minnow Medaka	Designed to detect (anti-) estrogenic and (anti-) androgenic effects. Animals are exposed to test chemical	[112] [113]
	Zebrafish	before the onset of sexual differentiation. Vitellogenin induction in males/inhibition in females.	[114]
		Gonadal histopathology	
		Hormone levels	
		Sex ratio	
		Development of intersex	
Fish Two	Fathead minnow	Whole body, serum, tissue T4	[115]
Generation Assay	Medaka	levels	
,, ,	Zebrafish		
Locomotion medium throughput assay (in Tier 2)	Zebrafish	Can identify subtle developmental abnormalities between the nervous and musculoskeletal systems	[116]
Sex specific behavior	Zebrafish	Sex specific behaviors (aggressive: nipping, chasing, circling, avoiding, and reproductive: female association, spawning, chasing, and nipping)	[117]
Short-term	Fathead minnow	Designed to detect (anti-)	[115], [118];
reproduction assay/ 21-day fish assay	Medaka	estrogenic and (anti-) androgenic effects. Mature male and female fish will be monitored during a 21-day chemical exposure; survival, reproductive behavior, and secondary sexual characteristics will be	[119], [120]

		observed while fecundity and fertilization success will be monitored daily. At termination of the assay, measurements will be made of a number of endpoints reflective of the status of the reproductive endocrine system, including the GSI, gonadal histology, and plasma concentrations of vitellogenin.	
Transgenic reporter lines	Zebrafish	Current lines can detect estrogenic activity and aromatase induction. More transgenic lines are being developed.	[41, 121, 122]
Assay	Amphibian species	Endpoints	Reference(s)
Corticosteroid secretion	X. laevis Rana catesbeiana	Corticosteroid secretion in response to ACTH	[123]
SEXDAMAX	X. laevis X. tropicalis	Sexual differentiation Metamorphosis	[124] [125]

Species	Family/	Adult Size	Generation	Sexually	Blood	Clutch size	Hatch time
	Distribution		Time	Dimorphic	Collection		
Fathead	Cyprinidae/ North	50 – 75mm	4 mos	Yes	Yes	50 – 200	4 – 5 days
minnow	America	2 – 5 g				every 3	
		-				days	
Japanese	Adrianichthyidae/	25 – 50mm	2 – 3 mos	Yes	No	10 – 30	8 – 10 days
medaka	Southeast Asia	0.7 – 0.8 g				daily	-
Zebrafish	Cyprinidae/ India	40 – 50mm	2 – 3 mos	Very little	A few	>150 every	2 – 3 days
	and Myanmar	1.5 g			microliters	5 – 10 days	

Table S6: Factors for considerationin fish EDC studies

Table S7: Selectin	ng species for amphibian assays	3
Species	Advantages	Disadvantages
Xenopus laevis	 Well-established laboratory model, with available molecular and endocrinology tools. Individual females can breed once per month, year round, and husbandry techniques are well- established. It responds to thyroid hormone, 	Some aspects of <i>X. laevis</i> biology may not be reflective of the majority of amphibians. For example, its putative sex- determining gene (DMW) is apparently unique even in the genus. Larvae are not sex-reversed by androgens as in other species.
	estrogen, and androgens. Females have large clutch sizes (2000 eggs and higher in large adults) so fully replicated experiments can be conducted easily.	Corticoids do not enhance larval development <i>in vivo</i> as in other species. Few amphibians are completely aquatic as adults.
	Aquatic throughout its life cycle so embryos, larvae and adults can be treated by immersion.	Generation time is about two years under ideal conditions, a long period for studies aimed at the full life cycle.
Hyperolius argus	Breeds repeatedly in the laboratory. Clear external markers for androgen, estrogen, and thyroid hormone effects. A single female may produce eggs once every few weeks. Breeding is spontaneous (unlike <i>X. laevis</i>) and does not require hormonal manipulation of adults.	Small clutch size (about 200). More complicated husbandry.
Lithobates pipiens	 <i>L. pipiens</i> is a well-studied species in the lab and the field. Widespread distribution in the northern U.S. allows for study in the field, along with closely related species in the southern US, some of which have ranges into Central America. In the laboratory, the sex ratio is 	Although newly collected females will breed in the laboratory, it is difficult to get them to cycle and produce regularly. There are no clear androgen or estrogen-dependent external markers at metamorphosis.

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affected by androgens and	
estrogens.	

Table S8: Examples of current assays, biological endpoints, and references, available for Tier 5 screening

Assay	Cell Type / Animal Model	Endpoints	Reference(s)
Asthma	Mouse	Pups (17 days old) are tested for	
Astrina	Mouse	functional markers of asthma:	[126]
		ELISA for IgE antibodies,	[120]
		eosinophilic inflammation (by	
		lavage) and airway hyper-	
		responsiveness by whole-body	
		barometric plethysmography.	
		Pregnant females are exposed to	
		xenoestrogens in their drinking	
		water. Pups (17 days old) are tested	
		for functional markers for asthma:	
		ELIZA for IgE antibodies,	
		eosinophilic inflammation (by	
		lavage) and	
		airway hyperresponsiveness by	
		whole-body barometric	
		plethysmography.	
Bone	Mouse	Fetuses are examined at embryonic	[127]
development		day 17 and calcification of the	
assay		bones is determined by alcian	
		blue/alizarin red incorporation	
Brain sexual	Mouse / Rat	Several regions of the brain are	[128-130]
dimorphism		known to have sex-differences in	
		the number and/or localization of	
		specific populations of neurons (i.e.	
		GABAergic cells, Tyrosine	
		Hydroxylase-positive cells, etc.)	
		Immunohistochemistry, in situ	
		hybridization and/or RT-PCR	
		analysis is used to measure these	
		differences in specific brain nuclei.	
Forced	Mouse	Females are paired with control	[131, 132]
Breeding Assay	medee	males (proven breeders) and 1) the	[101, 102]
Drooding/toody		time to mating is determined; 2) the	
		number of pups delivered is	
		determined.	
Kidney function	Rat	Blood urea nitrogen concentrations	[133, 134]
assay		are measured using standard	
ussay		diagnostic kits. Levels of	
		Malondialdehyde (a measure of	
		lipid peroxidation) and Glutathione	
		(an antioxidant) are measured in	
		kidney extracts.	
Mammany aland			
Mammary gland	Rat	Thin sections of mammary tissues	[135-138]
carcinogenesis		are examined for neoplastic lesions	
		(hyperplasias and DCIS) in animals	
		with and without exposures to sub-	
		effective doses of chemical	
Mommony alard	Mayoo pubarty & adulthead	carcinogens.	[64 66]
Mammary gland	Mouse – puberty & adulthood	Morphological characteristics of	[64-66]

development		whole mount mammary glands. In	
		pubertal animals, the number and density of TEBs (proliferative	
		structures) and size of the tree are	
		easily assessed. In adult animals,	
		the density of epithelial structures	
		(alveolar buds & terminal ends) are	
		calculated using a grid	
	Maria	superimposed on whole mounts.	[400]
Maternal	Mouse	Between birth and weaning, dams are assessed at several discrete	[139]
behavior assay		periods to determine time spent	
		with/away from, nursing, and	
		licking/grooming the pups.	
		Additional tests can include the time	
		it takes dams to retrieve pups that	
		are moved by an experimenter out	
		of the nest.	
Obesity/	Mouse / Rat	1) Body weight is monitored over	[140, 141]
metabolic		several months.	
syndrome		2) Fat deposition is determined	
assays		with CT-scan (live) and fat pad	
		dissection (at time of death) 3) Fasting glucose/insulin levels	
		are measured. Glucose and	
		insulin tolerance tests are also	
		performed.	
		4) Food consumption and activity	
		tests.	
		5) RT-PCR analysis of brown and	
		white fat.	
Prostate	Rat	Adult animals are treated with a	[142, 143]
carcinogenesis		cocktail of estrogen & testosterone and the incidence of PIN lesions are	
		determined from thin sections. This	
		should be coupled with	
		immunohistochemical analysis to	
		quantify changes in specific cell	
		types.	
Senesence	Mouse / Rat	1) Mice/rats are kept until later	[54-56]
(aging) assay		adulthood (9-12 months) and	
		then mated to determine	
		whether their reproductive axis	
		is still capable of responding.	
		This can be done in males and	
		females. 2) Examination of methylation	
		patterns in tissues where	
		epigenetic changes are	
		associated with aging (i.e. brain)	
		3) Estrous cyclicity is observed	
		throughout adulthood for	
		changing patterns from normal	
_		cycles	
Sexually	Mouse / Rat	A number of behaviors are different	[144-147]
dimorphic		between males and females	

behavior assays		including play behaviors, anxiety, exploratory behaviors, and other social interactions. These assays determine whether the normal sex- specific behaviors are retained. These tests can also be performed in castrated males, ovariectomized females, and adrenalectomized males & females to determine whether replacement with controlled levels of hormone can normalize abnormal behaviors.	
Tissue gene expression assay	Mouse / Rat	Hormone-sensitive genes have been identified for several tissues in rodents as well as other species. Simple RT-PCR analysis allows expression of these genes to be compared between exposed/unexposed individuals. Micro-array technology is also available for more widespread screening of a multitude of genes. Finally, epigenetic changes can be assessed for single genes of interest by examining methylation patterns.	[148-150]

Electronic Supplementary Material (ESI) for Green Chemistry This journal is C The Royal Society of Chemistry 2012

	Assay	BPA	Atrazine	Perfluorinated compounds	Phthalates	Organotins	Perchlorate
Tier 1							
	Chemical reactivity						
	Physiochemical properties				[151]		
	Docking modeling	[152]			[153]	[154]	
	QSAR	[155]				[156]	
Tier 2							
	Tox21 qHTS	[157]	[157]		[157]		
Tier 3							
	MCF7 cell proliferation assay	[158]		[159]	[160]		
	Prostate cancer cell proliferation assay, PSA assay	[161]				[162]	
	3T3-L1 adipogenesis assay	[163]			[163]	[89]	
	GH3 T-screen assay	[164]			[165]		
Tier 4							
	Zebrafish rapid developmental toxicity HTS	[107, 108]	[109]	[110, 111]		[40]	
	Aquatic EDC reporter assays	[42, 166, 167] (ER) [43] (TH)					

Table S9: Use of TiPED to detect endocrine disrupting activity of known EDCs

		[160]	[160]	[170]	[171]	[170]	
	Medaka and fathead minnow reproductive assays	[168]	[169]	[170]	[171]	[172]	
	Xenopus metamorphosis assay	[173]			[174]		[175]
	Xenopus sexual dimorphism assays	[176]	[177]		[178]		[179]
	Frog metamorphosis assay	[173]			[174]		[175]
	<i>H. argus</i> color change assay						
	Xenopus corticoid assay		[51]				
Tier 5					·	·	
	Asthma assay	[180]					
	Brain sexual dimorphism assay	[129]			[181]	[182]	
	Mammary carcinogenesis assay	[138]	[183]				
	Mammary gland morphology assay	[64]	[184]	[185, 186]	[187]		
	Maternal behavior assay	[139]				[188]	
	Obesity / Metabolic syndrome assays	[189, 190]	[191]		[192]	[89]	
	Prostate carcinogenesis assay	[193]					

dim	exual norphism havior assays	[129]					
ex	ssue gene pression say	[194]	[195]	[196]	[197]	[198]	[199]

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