SUPPLEMENTARY FIGURES



Dietary treatment

Supplementary Figure 1. Impact of dietary PFCs on enzyme markers of peroxisome proliferation. Mean rates of palmitoyl CoA oxidation (A) and catalase activity (B) are shown + SEM (N = 18 for control, N = 12 for E2, and N = 6 for all other treatments). The diet concentration was 250 ppm (approximately 5 mg/kg bw/day) for all perfluoroalkyl compounds and 5 ppm for E2, the positive control. *, p < 0.05 compared to the control treatment group as determined by one-way ANOVA with Dunnett's multiple comparisons post-hoc test.



Supplementary Figure 2. Saturation analysis of [³H]-E2 binding in juvenile rainbow trout liver. Binding characteristics of the estrogen receptor were compared in liver cytosol extracts obtained from juvenile rainbow trout fed control diet (A) or trout fed diet supplemented with 5 mg/kg E2 (B) for two weeks. Saturation plots of [³H]-E2 binding in trout liver cytosol extracts are shown, and each symbol represents the mean of triplicate observations. Total binding, TB; specific binding, SB; and non-specific binding, NSB. A non-linear regression model for one-site binding was used to calculate values for the dissociation constant (K_d) and maximum number of binding sites (B_{max}), which are as follows: control liver cytosol, $K_d = 4.4 \pm 0.7$ nM, $B_{max} = 136$ pM; E2 liver cytosol, $K_d = 4.3 \pm 0.4$ nM, $B_{max} = 151$ pM.

H.sapiens ESR1

O.mykiss eral

M.musculus Esr1





Supplementary Figure 3. Alignment of the ligand-binding domain amino acid sequences for human, mouse and trout ER α proteins. Protein sequence alignment was performed using ClustalW2 (EMBL-EBI) using the complete amino acid sequences for human ESR1 (accession P03372), mouse Esr1 (P19785), trout era1 (P16058) and trout era2 (Q0H7E4). This figure shows the relevant portion of the ligand-binding domain region (*e.g.*, amino acids 311 to 564 for human ESR1) and the putative sites of estrogen hydrogen-bonding at the indicated glutamic acid, arginine and histidine residues (shaded in black). Symbols in the last line indicate the level of similarity of the sequences: the star represents conserved residues, the colon indicates highly similar residues, and the dot indicates weakly similar residues.



Supplementary Figure 4. Superimposition of mouse and trout ER α homology models with the human ER α crystal structure. Overlay plots were created using the SuperImpose algorithm in ICM-Browser (Molsoft). In both panels, hER α is represented in gray, whereas mER α (A) is shown in red and rtER α 1 (B) is shown in blue. E2 is shown as a stick/space-filling model in both plots in the orientation determined by the hER α crystal structure (PDB accession 1ERE).







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(Previous page)

Supplementary Figure 5. In silico model showing docking of estrogens and select polyfluorinated chemicals into the mERa ligand-binding domain. Docking of test ligands into the mERa ligand-binding domain was performed using Molsoft ICM, and the lowest ICM-scored poses for each calculated ligand-protein docking are shown. Ligands are colored by atom type (the carbon atoms in tan) and are displayed as ball and sticks. Relevant protein residues are displayed as ball and sticks and colored by atom type: carbon atoms in gray, oxygen in red, sulfur in yellow and fluorine in cyan. Hydrogen bonds are represented by black dotted lines between the donor (D) and the acceptor (A) and are defined as follows: Distance D---A: 2.8-3.2 Å; Angle D-H---A: 140-180°.





(Previous page)

Supplementary Figure 6. In silico model showing docking of estrogens and select polyfluorinated chemicals into the rtERa1 ligand-binding domain. Docking of test ligands into the rtERa1 ligand-binding domain was performed using Molsoft ICM, and the lowest ICM-scored poses for each calculated ligand-protein docking are shown. Ligands are colored by atom type (the carbon atoms in tan) and are displayed as ball and sticks. Relevant protein residues are displayed as ball and sticks and colored by atom type: carbon atoms in gray, oxygen in red, sulfur in yellow and fluorine in cyan. Hydrogen bonds are represented by black dotted lines between the donor (D) and the acceptor (A) and are defined as follows: Distance D---A: 2.8-3.2 Å; Angle D-H---A: 140-180°.

		H2 (J
H.sapiens ESR1	LALSITADOMVSALLDAEPPILYSEYDPTRPFS	FASMMGLLTNLADR	359
M musculus Esrl		FASMMGLLTNLADRELVHMIN	363
O mykies eral			372
0 mykiss_crai			311
D. mamia agm1	GGGEGRERI IMFFEQVEFELQGAEFFALC35QQEGRFII		207
D.rerio_esri	GGVVSILCMSPDQVLLLLLGAEPPAVCSRQAHSRPII		211
H.Sapiens_ESR2	LSPEQLVLTLLEAEPPHVLISRPS-APFT	EASMMMSLTKLADK LVHMIS	311
M.Musculus_Esr2	LSPEQLVLTLLEAEPPNVLVSRPS-MPFT	EASMMMSLTKLADKELVHMIG	311
O.mykiss_erbl	L'I'PEQLIGRIMAAEPPEIFLQKDMRRPL'I	'EANVMMSL'I'NLADK D LVHMIS	346
O.mykiss_erb2	SLTPEQLISCIMEAEPPEIYLMEDLKKPFT	'EASMMMSLTNLADK <mark>B</mark> LVLMIS	349
D.rerio_esr2b	GRAEG-RALNYSPEQLVSCILEAEPPQIYLREPVKKPYT	'EASMMMSLTSLADK <mark>E</mark> LVLMIS	389
D.rerio_esr2a	LSPEELISRIMEAEPPEIYLMKDMKKPFT	EANVMMSLTNLADK	329
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	Н2 Н3		
H sapions FSP1			110
M museulus Farl	WARKEVIGE VILLINDOVILLECAWLET MICLYWROMEN	IPCKLI FADNILI DDNOCKCVE	419
M. Musculus_Esti	WARKVPGPGDLNLHDQVHLLECAWLEIMIGLVWNSMEH	IFGKLLFAFNLLLDRNQGKCVE	423
O.mykiss_eral	WARKVPGFQELSLHDQVQLLESSWLEV MIGLIWSIHC	PGKLIFAQDLILDRSEGDCVE	432
O.mykiss_eraz	WARKIPGFQELSLHGQVQLLESSWLEVMIGLIWRSIPS	PGKLIFAKDLILDRSEGDCVE	3/1
D.rerio_esrl	WAKKVPGFQDLSLHDQVQLLESSWLEV	PGKLIFAQDLILDRSEGECVE	387
H.sapiens_ESR2	WAKKIPGFVELSLFDQVRLLESCWMEV	IPGKLIFAPDLVLDRDEGKCVE	371
M.Musculus_Esr2	WAKKIPGFVELSLLDQVRLLESCWMEV	IPGKLIFAPDLVLDRDEGKCVE	371
O.mykiss_erb1	WAKKIPGFVDLCLFDQVHLLECCWLEV	IPGRLIFSPDLSLNREEGSCVQ	406
O.mykiss_erb2	WAKKIPGFVELSLTDQVHLLECCWLEV <mark>I</mark> MLGLMW <mark>R</mark> SVDH	PGKLIFSPDLKLNREEGNCVE	409
D.rerio_esr2b	WAKKIPGFVELTLSDQVHLLECCWLDI	IPGKLIFTPDLKLNREEGNCVE	449
D.rerio_esr2a	WAKKIPGFVELSLFDQVHLLECCWLEVIMLGLMWRSVNH	IPGKLIFSPDLCLSRDESSCVQ	389
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	нь нь нь	н7 ()	
H conjona ESP1			170
M. sapiens_Eski	GMVETEDMILATSSKERMMNLOGEEEVCLKSTILLNSGV	TIFLSSILKSLEEKDHINKVL	4/5
M. Musculus_Esti	GMVEIFDMLLAISSRERMMLLQGEEFVCLKSIILLNSGV	TIFLSSTLKSLEERDHINKVL	403
O. mykiss_erai	GMAEIFDMLLAIVSRFRMLKLKPEEFVCLKAIILLNSGA	PARAMETER INAPPLICANT	492
D.mykiss_eraz	GMAEIFDMLLAIVSRFRMLKLKPEEFVCLKAIILLNSGA	LF SFCC I SVESLHNSPEVQSML	431
D.rerio_esri	GMAEIFDMLLATVARFRSLKLKLEEFVCLKAIILINSGA	LFSFCSSPVEPLMDNFMVQCML	44/
H.sapiens_ESR2	GILEIFDMLLATTSRFRELKLQHKEYLCVKAMILLNSSM	IYP-LVTATQDADSSRKLAHLL	430
M.Musculus_Esr2	GILEIFGMLLATTARFRELKLQHKEYLCVKAMILLNSSM	IYP-LATASQEAESSRKLTHLL	430
O.mykiss_erbl	GFVDIFDMLLAATSRFRELKLQREEYVCLKAMILLNSNM	ICLSSSEGSEELQSRSKLLRLL	466
O.mykiss_erb2	GIMEIFDMLLAATSRFRELNLQREEYVCLKAMILLNSNI	CSNSPERAEDLESRGKLLRLL	469
D.rerio_esr2b	GIMEIFDMLLATTSRFRELKLQREEYVCLKAMILLNSNN	ICSSLPQTPEDVESRGKVLNLL	509
D.rerio_esr2a	GLVEIFDMLLAATSRFRELKLQREEYVCLKAMILLNSNM	ICLGSSEGGEDLQSRSKLLCLL	449
	*: :**.***: :*** ::*: :*:*:*:*:*:**:**	: . : :*	
	Н7 ()— Н8	0	
H. sapiens ESB1	DKITDTLIHLMAKAGLTLOOOHORLAOLLLILSHIRHMS		539
M.musculus Esrl	DKTTDTLTHLMAKAGLTLQQQHQKLAQLLLLLSHTRHMS	NKGME	543
O mykies eral		NKCME IVSTKCKNKVPLVDI.	552
0 mykiss era?			491
D rerio esrl		NKCMENI VRMKCKNRVPLVDI.	507
H canione FCD?	NYALDYI'MMALYKGU GGUUGWDI YMI I WI I GRADDAG DYFI DYFI I GIOLOGYDI GRAGUNGWANNNI I WI I GRADDAG		101
M Mucculus Ear?			490
M.MUSCUIUS_ESIZ	NAVIDALVWVISASGISSQQQSVKLANLLMLLSHVKHIS		490
O.mykiss_erbi	DAVIDALVWAIAKIGLSPQQQSAKLAHLLMLLSHIRHVS		520
U.mykiss_erb2	USVIDALVWAISKKGLSPQQQSSRLAHLLMLLSHIRHVS		529
D.rerio_esr2b	DSVTDALVWIISRTGLSSQQQSIRLAHLLMLLSHIRHLS	NKGIELSNMKRKNVVLLYDL	569
D.rerio_esr2a	DSVTDALVWAISKTGLSFQQRSTRLAHLLMLLSHIRHVS	NKGMULHCMKMKKMAPLYDL	509
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Supplementary Figure 7. Alignment of the ligand-binding domain amino acid sequences for human, mouse, trout and zebrafish ER α and β isoforms. Protein sequence alignment was performed using ClustalW2 (EMBL-EBI) using the complete amino acid sequences for human ESR1 (accession P03372), mouse Esr1 (P19785), trout era1 (P16058), trout era2 (Q0H7E4), zebrafish esr1 (P57717), human ESR2 (Q92731), mouse Esr2 (O08537), trout erb1 (Q0H7E3), trout erb2 (Q0H3B4), zebrafish esr2a (Q8AV62) and zebrafish esr2b (Q90WS9). Zebrafish sequences have been included as this fish model is now commonly used for toxicity testing. This figure shows the relevant portion of the ligand-binding domain region (*e.g.*, amino acids 311 to 564 for human ESR1) and the putative sites of estrogen hydrogenbonding for ER α and corresponding alignment with ER β proteins at the indicated glutamic acid, arginine and histidine residues (shaded in black). Symbols in the last line indicate the level of similarity of the sequences: the star represents conserved residues, the colon indicates highly similar residues, and the dot indicates weakly similar residues.