Report Summary

Indigo Biosciences Study Report
110407-1

3M ToxDocs
11-108

Prepared by:

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BACKGROUND:

Various toxicological studies in rodents have shown that perfluoroalkyl acids (PFAs) produce responses that are consistent with activation of the xenosensor nuclear receptors peroxisome proliferator activated receptor α (PPARα), constitutive androstane receptor (CAR), and pregnane X receptor (PXR) (Bjork and Wallace 2009; Curran et al. 2008; Martin et al. 2007; Pieterman et al. 2007; Ren et al. 2009; Rosen et al. 2010; Rosen et al. 2009; Shipley et al. 2004; Takacs and Abbott 2007; Vanden Heuvel et al. 2006; Wolf et al. 2008). The activations of these xenosensor nuclear receptors can lead to hepatocellular hyperplastic responses in rodents. Even though human hepatocytes contain active forms of PPARα and CAR/PXR receptors, there are significant differences exist in pharmacokinetic / pharmacodynamic profiles between the human and rodent forms. To date, a number of studies have shown that the hyperplastic component of the response to inducers of xenosensor nuclear receptors observed in rodents appears unlikely to be a component of the human hepatic response (Ross et al. 2010). In the evaluation of PFAs of various chain lengths, Wolf et al. (2008) demonstrated that activation of mouse PPARα was generally higher compared to that of human PPARα using transfected COS-1 cells. Bjork and Wallace (2009) also reported that primary rat hepatocytes were more effective in eliciting PPARα–related responses than primary human hepatocytes. Furthermore, with the 8-carbon perfluoroalkyls perfluorooctanesulfonate(PFOS) and perfluorooctanoate (PFOA), Bjork et al. (2011) showed that while multiple xenosensor nuclear receptors (i.e., PPARα, CAR, PXR) participate in the metabolic response to PFOA and PFOS exposures in both human and rat hepatocytes, however, the changes in primary human hepatocytes were more subtle and possibly reflect an adaptive metabolic response rather than an overt metabolic regulation observed in rodents.

OBJECTIVE:

The objective of the work reported herein was to investigate the effects of perfluorobutyrate (PFBA), PFOA, perfluorobutanesulfonate (PFBS), pherfluorohexanesulfonate (PFHxS), and PFOS on the activation of three human xenosensor nuclear receptors (thyroid receptors alpha (TRα), PXR, and CAR3) using HEK-293T transactivated cells.

MATERIALS AND METHODS:

The test was performed by Indigo Biosciences. The compounds and concentrations evaluated are:

Compound	Lot #	Concentrations Tested
PFBA, potassium salt	NB # 119868-94	0.41 – 100 µM
PFOA, ammonium salt	(FC-143) Lot # 332	0.41 – 100 µM
PFBS, potassium salt	(L-7038) Lot # 2	0.41 – 100 µM
PFHxS, potassium salt	(L-9051) NB # 118993-109	0.41 – 100 µM
PFOS, potassium salt	(FC-95) Lot # 217	0.41 – 100 µM
PFOS, potassium salt	(FC-95) TCR 00017-46	0.41 – 100 µM

RESULTS:

The full report from Indigo Biosciences is attached as Appendix I of this report. The table below summarizes the study results.

Xenosensor Nuclear Receptor	Compound	Dose Range (µM)	Highest Peak Response	EC50 (µM)	p-value
TRα	T3[a] (+ control)	0 - 0.01	80.43	0.002123	--
	PFOS	0.41 - 100	1.36	0.9528	0.0087
	PFOS (lot 217)	0.41 - 100	1.475	7.466	0.0014
	PFHxS	0.41 - 100	1.623	17.46	0.0015
	PFBS	0.41 - 100	1.224	2.534	0.0035
	PFOA	0.41 - 100	1.542	NA[b]	0.1817
	PFBA	0.41 - 100	0.978	1.758	0.3298
PXR	TO-9013173[c] (+ control)	0 - 1	8.343	0.03377	--
	PFOS	0.41 - 100	1.706	8.172	0.0032
	PFOS (lot 217)	0.41 - 100	2.045	122.2	0.0018
	PFHxS	0.41 - 100	2.81	47.25	0.0005
	PFBS	0.41 - 100	1.693	18.28	0.0008
	PFOA	0.41 - 100	1.07	NA	0.0765
	PFBA	0.41 - 100	0.966	NA	0.0314
CAR3	CITCO[d] (+ control)	0 - 5	37.042	0.6444	--
	PFOS	0.41 - 100	2.025	38.11	0.0001
	PFOS (lot 217)	0.41 - 100	2.547	525.9	0.0021
	PFHxS	0.41 - 100	1.963	62.41	0.0516
	PFBS	0.41 - 100	1.13	3.247	0.0525
	PFOA	0.41 - 100	1.723	NA	0.0275
	PFBA	0.41 - 100	1.15	3.885	0.128

[a] T3, triiodothyronine, agonist for TRα.
[b] NA, not available, EC_{50} cannot be derived because data did not fit the curve.
[c] TO-9013173, agonist for PXR.
[d] CITCO, 6-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehydeO-(3,4-dichlorobenzyl)oxime, agonist for CAR3.

Human thyriod receptor α (TRα):

There were statistical associations suggesting that PFBS, PFHxS, and PFOS were capable of eliciting human TRα activation in the assay. However, unlike the positive control triiodothyronine (T3), there were no concentration-dependent responses with any of the PFAs evaluated in the activation of human TRα and the EC50's were at least several orders of magnitude higher than T3. Therefore, PFAs does not have any effect on TRα activation.

Human pregnane X receptor (PXR):

Within the limit of study design, PFOS (lot 217) and PFHxS at 33 μM or higher appeared to elicit some human PXR responses, although the EC50's derived were several orders of magnitude higher than the positive control. PFBS did not have any effect on PXR activation.

Even though the data obtained from PFOA and PFBA did not fit the statistical curves and no reliable EC50 could be derived, given the fact that the highest responses obtained with either PFOA or PFBA were at least an order of magnitude lower than the highest response obtained from positive control, it is unlikely that PFOA or PFBA is capable of activating human PXR.

Human Constitutive androstane receptors 3 (CAR3):

PFOS (both lot 217 and TCR 00017-46) at 100 μM (the highest concentration tested) were capable of eliciting human CAR3 responses. Similar to PXR, the EC50's derived were several orders of magnitude higher than the positive control. PFBS and PFBA did not have any effect on human CAR3 activation.

Due to the lack of concentration-dependent response, the data obtained from PFOA did not fit the statistical curves and no reliable EC50 could be derived, given the fact that the highest response obtained were much lower than the highest response obtained from positive control, it is unlikely that PFOA activates human CAR3.

CONCLUSION:

PFAs do not appear to activate human TRα. Consistent with other study data reported by others, PFOS appears to be capable of eliciting human PXR and CAR3 responses *in vitro*. However, the degree of activation was several folds lower than the activation produced by TO-9013173 and CITCO (the respective controls for PXR and CAR3), and the concentrations of PFOS employed in the assay were several folds higher than the blood serum PFOS concentrations reported for general human population. PFHxS also appears to be capable of eliciting human PXR responses as well, and similar to PFOS, the extent of activation was much lower than the respective control and the concentrations of PFHxS that produced effects were much higher than the general human population. PFBS, PFOA, and PFBA did not activate human PXR and CAR3.

REFERENCES:

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Wolf, C.J.,Takacs, M.L.,Schmid, J.E.,Lau, C., Abbott, B.D., 2008. Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicol Sci 106, 162-171.

APPENDIX I: Study Report from Indigo Biosciences

Indigo Biosciences, Inc

Report 110407-1

3M

Summary

April 7, 2011

SUMMARY

Effects of test compounds on nuclear receptor activation:

- TRalpha: No effects
- PXR: PFOS217 at 100 µM; PFHxS at 33 and 100 µM
- CAR3: PFOS at 100 µM; PFOS217 at 100 µM

EXPERIMENTAL PROCEDURE

Plasmids:

Two different assays are commonly used for services, a chimeric assay and a full- length (see Figure 1).

Chimeric System: The ligand-binding domain of the nuclear receptors were fused to the DNA-binding domain of the yeast transcription factor Gal4 under the control of the SV40 promoter. A reporter plasmid encodes the firefly luciferase gene under the control of the Gal4 DNA response element (UAS). A transfection efficiency control vector is included in most assays (pRL-luciferase, Promega, Madison, WI). All plasmids were verified by sequencing and through examination of positive controls (Tien et al., 2006; Vanden Heuvel et al., 2006).

Full Length System: The full-length cDNA of the nuclear receptor is under the control of the SV40 promoter. A reporter plasmid encodes the luciferase reporter under the control of the MMTV response element. All plasmids were verified by sequencing and through examination of positive controls.

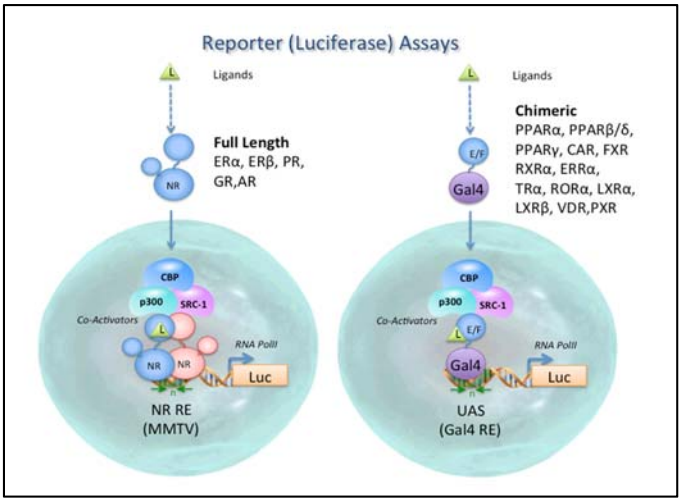

Figure 1. Design of Reporter Assays Performed by INDIGO Biosciences, Inc.

Cell culture and transactivation assays:

HEK 293-T fibroblasts (ATCC, Manassas, VA) were cultured in high-glucose Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Sigma), 0.2 mg/ml streptomycin and 200 U/ml penicillin (Gibco, Grand Island, NY). For transient transfection reporter assays, HEK 293-T ells were transfected with plasmid DNA using Lipofectamine reagent (Invitrogen, Carlsbad, CA) and following the manufacturer's recommended procedures, using HEK 293-T cells at approximately 80% confluence in 10 cm culture dishes. After 6 h, the DNA- Lipofectamine complex was removed. Following overnight culture, the media was replaced 4 h after replating with DMEM (10% FBS) containing test compounds in DMSO (0.1% final concentration). Concentrations of the chemicals are given in the figure legends. Sixteen hours after treatment, the cells were lysed with passive lysis buffer (Promega, Madison, WI) for 30 min; luciferase activity was measured using the Luciferase dual reporter assay kit (Promega, Madison, WI) and a Tecan GeniosPro (Research Triangle Park, NC) and manufacturer's recommended procedures. The fold induction of normalized luciferase activity (luciferase/ renilla) was calculated relative to vehicle-treated cells, and represents the mean of three independent samples per treatment group.

Statistical Analysis:

Significance of dose-dependent alterations in relative luciferase activity ($p<0.05$) was determined using ANOVA ($p<0.05$, JMP 7.0, SAS Institute, Cary,NC). Dunnett's multi-comparison t-test was used to determine doses that resulted in significant differences relative to vehicle control treated cells (JMP 7.0). Non-linear regression was performed using Prism 5.0 (Graphpad Software, La Jola, CA).

References:

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Results:

(A) Human TRalpha Activity Assay

Human TRalpha Activity Assay

RAW DATA						SUMMARIZED DATA								
Group	Compound	Dose[1]	Luc1[2]	Luc2	Luc3	Group	Compound	Dose	Mean[3]	Std Err	CV	Stats[4]	p-value[5]	Renilla[6]
1	DMSO	0	0.7829	1.2260	1.0826	1	DMSO	0	1.0000	0.0398	6.8851			
2	T3	3.2	1.3911	1.4683	1.2120	2	T3	3.2	1.1945	0.0668	9.6853			
3	T3	16	1.6808	1.7300	1.6432	3	T3	16	1.4827	0.0221	2.5819			
4	T3	80	2.8709	2.4996	2.3047	4	T3	80	2.2518	0.1462	11.2433			
5	T3	400	13.4186	13.5485	14.5054	5	T3	400	12.1671	0.3016	4.2934			
6	T3	2000	49.8262	57.6902	62.8881	6	T3	2000	49.9929	3.3416	11.5774			
7	T3	10000	97.6308	88.6182	87.9161	7	T3	10000	80.4340	2.7529	5.9280			
8	PFOS	0.41	1.5336	1.1860	1.2438	8	PFOS	0.41	0.9704	0.0790	14.0966	0.0087	0.9993	0.90724986
9	PFOS	1.23	1.6208	1.3368	1.2753	9	PFOS	1.23	1.0364	0.0782	13.0617		0.9978	1.00495683
10	PFOS	3.7	1.7300	1.6780	1.6351	10	PFOS	3.7	1.2348	0.0202	2.8275		0.1273	0.92482502
11	PFOS	11.1	1.4320	1.8906	1.7369	11	PFOS	11.1	1.2388	0.0990	13.8422		0.1186	0.78996843
12	PFOS	33.3	2.0059	1.7053	1.8423	12	PFOS	33.3	1.3598	0.0638	8.1285		0.012	0.5186502
13	PFOS	100	1.3598	1.5801	1.3423	13	PFOS	100	1.0485	0.0562	9.2833		0.9904	0.61092297
14	PFOS217	0	1.4301	1.4621	1.1920	14	PFOS217	0	1.0000	0.0626	10.8415	0.0014	1	1
15	PFOS217	0.41	1.4540	1.4147	1.3160	15	PFOS217	0.41	1.0246	0.0302	5.0976		0.9996	1.00549124
16	PFOS217	1.23	1.3263	1.3898	1.5206	16	PFOS217	1.23	1.0373	0.0420	7.0181		0.9963	1.06633562
17	PFOS217	3.7	1.4341	1.6616	1.6981	17	PFOS217	3.7	1.1738	0.0607	8.9535		0.2795	0.94738703
18	PFOS217	11.1	1.1855	1.6524	1.6048	18	PFOS217	11.1	1.0878	0.1090	17.3490		0.837	0.82781468
19	PFOS217	33.3	2.0159	2.1002	1.9061	19	PFOS217	33.3	1.4745	0.0413	4.8480		0.0007	0.65097695
20	PFOS217	100	1.7592	1.8390	1.5170	20	PFOS217	100	1.2524	0.0711	9.8333		0.0641	0.63612893
21	PFHxS	0	1.0655	1.2300	0.9756	21	PFHxS	0	1.0000	0.0683	11.8314	0.0015	1	1
22	PFHxS	0.41	1.3552	1.1638	1.2255	22	PFHxS	0.41	1.1447	0.0517	7.8284		0.7995	0.98896434
23	PFHxS	1.23	1.2197	1.1961	0.9992	23	PFHxS	1.23	1.0440	0.0641	10.6380		0.9991	0.99012433
24	PFHxS	3.7	1.02835	1.223711	1.431821	24	PFHxS	3.7	1.12618328	0.10683624	16.4312322		0.8723	0.99138284
25	PFHxS	11.1	1.392872	1.459168	1.302646	25	PFHxS	11.1	1.27011069	0.04160013	5.67301272		0.2744	1.02595598
26	PFHxS	33.3	1.623792	1.799535	1.884285	26	PFHxS	33.3	1.6225666	0.07035373	7.51009139		0.0028	0.79998882
27	PFHxS	100	1.75969	1.39303	2.142978	27	PFHxS	100	1.61892442	0.1985641	21.243926		0.0029	0.54180085
28	PFBS	0	1.035781	1.261699	1.234255	28	PFBS	0	1	0.06045835	10.4716941	0.0035	1	1
29	PFBS	0.41	1.004704	1.079429	1.217323	29	PFBS	0.41	0.9347972	0.05289827	9.80132274		0.9307	0.8384736
30	PFBS	1.23	0.7317497	1.077492	1.036778	30	PFBS	1.23	0.8058418	0.0926712	19.9184531		0.1556	0.66056222
31	PFBS	3.7	1.280438	1.577579	1.464072	31	PFBS	3.7	1.22378633	0.07353596	10.4077002		0.0849	0.83033015
32	PFBS	11.1	1.188956	1.45895	1.279778	32	PFBS	11.1	1.11211175	0.06737708	10.4935965		0.6166	0.92584538
33	PFBS	33.3	1.347046	1.306689	1.344105	33	PFBS	33.3	1.13197621	0.01103419	1.68835449		0.4681	0.85057157
34	PFBS	100	1.301196	1.379461	1.349161	34	PFBS	100	1.14103068	0.01935382	2.93785192		0.4068	0.69897459
35	PFOA	0	1.208199	1.082189	1.120016	35	PFOA	0	1	0.03283914	5.68790617	0.1817	1	1
36	PFOA	0.41	1.162493	1.788925	1.337093	36	PFOA	0.41	1.25747888	0.16418436	22.6147452		0.5882	1.11237482
37	PFOA	1.23	1.45962	1.604089	1.301355	37	PFOA	1.23	1.27992578	0.07690176	10.4066779		0.5121	1.24511386
38	PFOA	3.7	1.917233	1.412162	1.929093	38	PFOA	3.7	1.54189592	0.14986618	16.8348481		0.0555	1.1221669
39	PFOA	11.1	1.41738	1.93721	1.387912	39	PFOA	11.1	1.3905983	0.15692358	19.5455164		0.2224	1.15636803
40	PFOA	33.3	1.250512	1.199197	1.943313	40	PFOA	33.3	1.28812364	0.21106931	28.3810304		0.4853	0.98125502
41	PFOA	100	1.214905	1.29889	1.253875	41	PFOA	100	1.10475768	0.02134525	3.34653057		0.9835	0.67508295
42	PFBA	0	1.498251	1.936381	1.693365	42	PFBA	0	1	0.07413945	12.84133	0.3298	1	1
43	PFBA	0.41	1.694401	1.747789	1.292758	43	PFBA	0.41	0.92335233	0.08401431	15.7596455		0.8183	1.01709165
44	PFBA	1.23	1.639416	1.554529	1.692198	44	PFBA	1.23	0.95283656	0.02345958	4.26444437		0.9727	1.14804843
45	PFBA	3.7	1.778415	1.648278	1.589801	45	PFBA	3.7	0.97825603	0.03261079	5.77390251		0.9995	1.02335903
46	PFBA	11.1	1.413671	1.450736	1.361416	46	PFBA	11.1	0.82406893	0.01515706	3.18575257		0.1511	1.00881868
47	PFBA	33.3	1.434516	1.443324	1.613287	47	PFBA	33.3	0.87580531	0.03403547	6.73108011		0.4261	1.12207964
48	PFBA	100	1.662825	1.405156	1.822196	48	PFBA	100	0.95362322	0.07107962	12.9100795		0.9747	1.094378

[1] Dose in pM for T3 and uM for client's compounds
[2] Expressed as (Luciferase RLU/Renilla RLU)
[3] Expressed relative to DMSO control
[4] Oneway Anova, Dose x Relative Luciferase, Prob > F
[5] Dunnett's t-test, DMSO control versus each sample
[6] Relative Renilla activity (toxicity marker)
Relative renilla below 50% (0.5) indicates overt toxicit

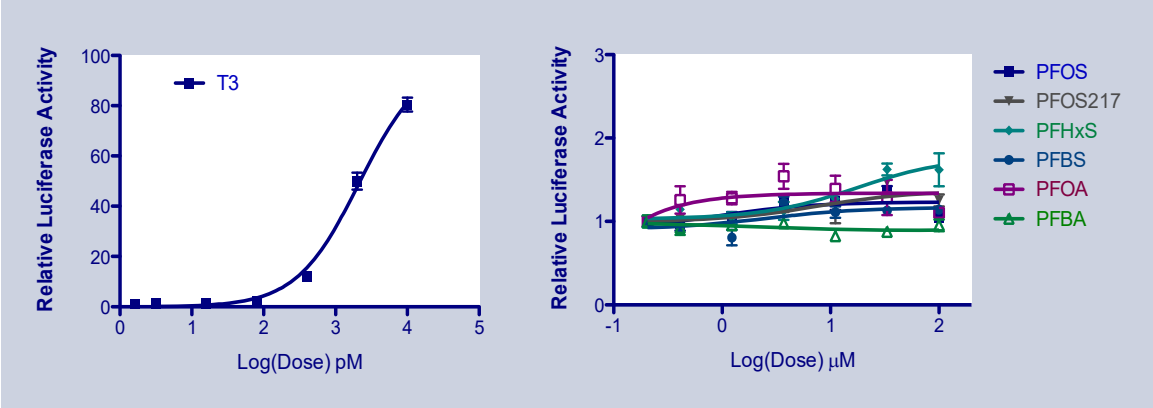
Curve Fitting Data

	T3	PFOS	PFOS217	PFHxS	PFBS	PFOA	PFBA
Bottom	-0.07873	0.9065	0.9974	1.028	0.9063	Ambiguous	0.9826
Top	98.35	1.233	1.366	1.779	1.167		0.894
LogEC50	3.327	-0.02099	0.8731	1.242	0.4038		0.245
EC50	2123	0.9528	7.466	17.46	2.534		1.758
Span	98.43	0.327	0.369	0.7513	0.261		-0.08855

Statistical Analysis

		PFOS	PFOS217	PFHxS	PFBS	PFOA	PFBA
P-value		0.0087	0.0014	0.0015	0.0035	0.1817	0.3298
Peak[4]		1.360	1.475	1.6225666	1.2237863	1.5418959	0.8240689
LSSD[5]		33.3	33.3	33.3	N/A	N/A	N/A

[4] Highest observable response (relative to DMSO control)
[5] Lowest statistically significant dose (p<0.05, Dunnett's)
NA, not applicable



(B) Human PXR Activity Assay

Human PXR Activity Assay

RAW DATA						SUMMARIZED DATA								
Group	Compound	Dose[1]	Luc1[2]	Luc2	Luc3	Group	Compound	Dose	Mean[3]	Std Err	CV	Stats[4]	p-value[5]	Renilla[6]
1	DMSO	0	0.2699435	0.7931641	0.9932408	1	DMSO	0	1	0.06773475	11.7320024			
2	TO-9013173	0.32	1.423668	2.339388	1.035379	2	TO-9013173	0.32	1.82006755	0.43987464	41.8602721			
3	TO-9013173	1.6	2.186356	1.62824	1.885377	3	TO-9013173	1.6	2.16202489	0.18352244	14.7024301			
4	TO-9013173	8	2.075191	2.24288	2.516727	4	TO-9013173	8	2.59246901	0.14642964	9.78308966			
5	TO-9013173	40	4.315969	4.973182	4.966083	5	TO-9013173	40	5.40707309	0.24794841	7.94254565			
6	TO-9013173	200	7.138336	6.818035	7.072407	6	TO-9013173	200	7.97630819	0.11111887	2.41293994			
7	TO-9013173	1000	6.916809	6.96418	8.114037	7	TO-9013173	1000	8.34281032	0.44540175	9.24698541			
8	PFOS	0.41	1.451643	1.376961	1.484295	8	PFOS	0.41	0.85179388	0.01882165	3.82722316	0.0032	0.9165	0.907249863
9	PFOS	1.23	1.790085	1.547474	1.778647	9	PFOS	1.23	1.01044632	0.04682685	8.02679824		1	1.004956829
10	PFOS	3.7	1.87229	2.037152	1.930471	10	PFOS	3.7	1.15337784	0.02860172	4.29517767		0.9048	0.924825023
11	PFOS	11.1	1.613219	3.017765	2.610429	11	PFOS	11.1	1.43017289	0.24719367	29.9370795		0.1429	0.789968427
12	PFOS	33.3	2.22789	2.980243	2.454123	12	PFOS	33.3	1.51328903	0.13204547	15.113403		0.0644	0.518650202
13	PFOS	100	2.704702	3.347376	2.587978	13	PFOS	100	1.70640369	0.13988599	14.1988466		0.009	0.610922973
14	PFOS217	0	1.913311	1.918895	1.231107	14	PFOS217	0	1	0.1352895	23.4328283	0.0018	1	1
15	PFOS217	0.41	1.616936	1.877738	1.365523	15	PFOS217	0.41	0.95988476	0.08761379	15.8093494		1	1.005491244
16	PFOS217	1.23	1.555113	2.457464	2.152485	16	PFOS217	1.23	1.21759449	0.1570151	22.3356905		0.7988	1.066335615
17	PFOS217	3.7	2.453635	2.57857	2.489945	17	PFOS217	3.7	1.48561821	0.0219844	2.56311428		0.1484	0.947387028
18	PFOS217	11.1	1.455274	2.340294	2.048763	18	PFOS217	11.1	1.15425039	0.1542821	23.1513406		0.9419	0.827814675
19	PFOS217	33.3	2.625709	3.331317	1.913984	19	PFOS217	33.3	1.55451776	0.24242037	27.0105882		0.0838	0.650976949
20	PFOS217	100	3.184645	3.218487	3.950592	20	PFOS217	100	2.04485166	0.1480452	12.539873		0.0011	0.636128925
21	PFHxS	0	1.519225	1.242754	0.9651626	21	PFHxS	0	1	0.12874006	22.2984328	0.0005	1	1
22	PFHxS	0.41	1.513617	1.4925	1.631546	22	PFHxS	0.41	1.24429482	0.03482091	4.84704883		0.9358	0.98896434
23	PFHxS	1.23	1.227581	1.156105	1.445242	23	PFHxS	1.23	1.02730951	0.06998658	11.799785		1	0.990124327
24	PFHxS	3.7	0.9261827	1.522918	1.737681	24	PFHxS	3.7	1.12332241	0.19539558	30.1280445		0.9976	0.991382842
25	PFHxS	11.1	2.194648	2.146304	1.748068	25	PFHxS	11.1	1.63369699	0.11388829	12.0744727		0.2659	1.025955982
26	PFHxS	33.3	2.512845	2.365294	2.881724	26	PFHxS	33.3	2.08198771	0.12361465	10.283771		0.0233	0.799988824
27	PFHxS	100	2.747372	2.917912	4.808423	27	PFHxS	100	2.81011781	0.53158522	32.7649113		0.0004	0.541800852
28	PFBS	0	1.234832	1.597554	1.371582	28	PFBS	0	1	0.07547117	13.0719904	0.0008	1	1
29	PFBS	0.41	2.301728	2.410897	2.404659	29	PFBS	0.41	1.69299195	0.02525888	2.58416215		0.0021	0.838473597
30	PFBS	1.23	2.631239	1.983705	1.791228	30	PFBS	1.23	1.52383938	0.18131024	20.6083762		0.0173	0.660562222
31	PFBS	3.7	1.931713	1.97877	1.221857	31	PFBS	3.7	1.22083232	0.17471967	24.7882807		0.5181	0.830330148
32	PFBS	11.1	1.639419	1.663126	1.736557	32	PFBS	11.1	1.19865375	0.02086622	3.01516253		0.6136	0.925845375
33	PFBS	33.3	1.359096	1.397776	1.278307	33	PFBS	33.3	0.95985008	0.02511526	4.53205125		0.9997	0.850571568
34	PFBS	100	1.097309	1.191628	1.527382	34	PFBS	100	0.90778974	0.09313316	17.7696828		0.9732	0.69897459
35	PFOA	0	2.475862	2.712058	1.992268	35	PFOA	0	1	0.08850901	15.3302107	0.0765	1	1
36	PFOA	0.41	0.8823314	1.735937	1.606417	36	PFOA	0.41	0.58838089	0.11096929	32.666671		0.1224	1.112374818
37	PFOA	1.23	2.016315	1.389305	0.9668043	37	PFOA	1.23	0.6089568	0.12738341	36.2315586		0.1506	1.245113855
38	PFOA	3.7	1.732709	2.11884	1.785514	38	PFOA	3.7	0.78508571	0.05050336	11.1420164		0.6516	1.122166898
39	PFOA	11.1	1.730784	1.94652	2.246388	39	PFOA	11.1	0.82500514	0.062464	13.1139568		0.8018	1.156368033
40	PFOA	33.3	1.061295	1.556958	2.084046	40	PFOA	33.3	0.65489915	0.12337667	32.6301639		0.2345	0.981255019
41	PFOA	100	3.558488	2.108	2.014824	41	PFOA	100	1.0697926	0.2088036	33.8064073		0.9963	0.675082953
42	PFBA	0	1.737606	1.728782	1.955788	42	PFBA	0	1	0.04107671	7.11469436	0.031409	1	1
43	PFBA	0.41	1.564718	1.921795	1.171592	43	PFBA	0.41	0.85908407	0.1198679	24.1672842		0.702	1.017091648
44	PFBA	1.23	1.315794	1.614791	1.825245	44	PFBA	1.23	0.87710727	0.08177771	16.1488967		0.798	1.148048428
45	PFBA	3.7	1.205181	1.655908	1.55435	45	PFBA	3.7	0.81432971	0.07552401	16.0636932		0.4594	1.023359028
46	PFBA	11.1	1.082178	1.196779	1.458556	46	PFBA	11.1	0.68930131	0.06162773	15.4855875		0.0864	1.008818678
47	PFBA	33.3	1.451322	1.487678	1.59692	47	PFBA	33.3	0.83654975	0.02420658	5.01189864		0.5767	1.122079641
48	PFBA	100	1.919343	1.283868	2.034257	48	PFBA	100	0.96593471	0.12910719	23.1506552		0.9995	1.094378002

[1] Dose in nM for TO901317 and uM for client's compounds

[2] Expressed as (Luciferase RLU/Renilla RLU)

[3] Expressed relative to DMSO control

[4] Oneway Anova, Dose x Relative Luciferase, Prob > F

[5] Dunnett's t-test, DMSO control versus each sample

[6] Relative Renilla activity (toxicity marker)

Relative renilla below 50% (0.5) indicates overt toxicity

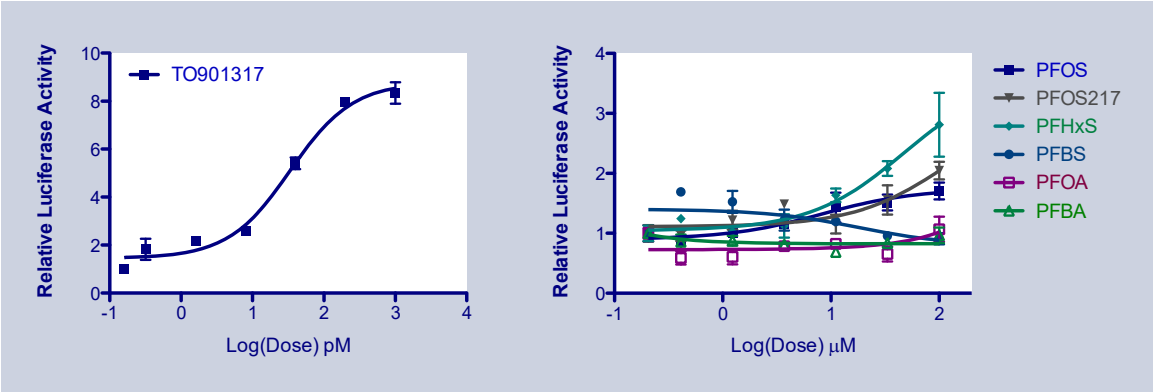
Curve Fitting Data							
	TO901317	**PFOS**	**PFOS217**	**PFHxS**	**PFBS**	**PFOA**	**PFBA**
Bottom	1.449	0.8992	1.111	1.044	1.401	Interrupted	Interrupted
Top	8.773	1.741	3.179	3.636	0.7898		
LogEC50	1.529	0.9124	2.087	1.674	1.262		
EC50	33.77	8.172	122.2	47.25	18.28		
Span	7.325	0.8413	2.068	2.591	-0.6114		

Statistical Analysis							
		PFOS	**PFOS217**	**PFHxS**	**PFBS**	**PFOA**	**PFBA**
P-value		0.0032	0.0018	0.0005	0.0008	0.0765	0.031409
Peak[4]		1.706	2.045	2.8101178	1.692992	1.0697926	0.6893013
LSSD[5]		100	100	33.3	0.41	N/A	N/A

[4] Highest observable response (relative to DMSO control)

[5] Lowest statistically significant dose (p<0.05, Dunnett's)

NA, not applicable



(C) Human CAR3 Activity Assay

Human CAR Activity Assay

RAW DATA						SUMMARIZED DATA								
Group	Compound	Dose[1]	Luc1[2]	Luc2	Luc3	Group	Compound	Dose	Mean[3]	Std Err	CV	Stats[4]	p-value[5]	Renilla[6]
1	DMSO	0	0.2167248	0.6569583	0.9400501	1	DMSO	0	1	0.10351685	17.9296436			
2	CITCO	1.6	1.506333	2.135472	1.036676	2	CITCO	1.6	1.89641876	0.3870763	35.3527305			
3	CITCO	8	1.661328	1.75017	1.654854	3	CITCO	8	2.05364197	0.03739328	3.15376591			
4	CITCO	40	1.616416	1.645852	2.143853	4	CITCO	40	2.19136708	0.20808699	16.4471411			
5	CITCO	200	6.079296	7.039314	6.824971	5	CITCO	200	8.08411556	0.35379451	7.58017458			
6	CITCO	1000	26.20632	22.4475	22.89514	6	CITCO	1000	29.0023171	1.4415003	8.60880098			
7	CITCO	5000	29.71467	30.46399	31.20507	7	CITCO	5000	37.0423262	0.52319677	2.44639979			
8	PFOS	0.41	1.2954	1.461348	1.263326	8	PFOS	0.41	0.81192113	0.03718058	7.93163981	0.0001	0.6929	0.907249863
9	PFOS	1.23	1.48921	1.626203	1.499532	9	PFOS	1.23	0.93206527	0.02668682	4.95919338		0.9951	1.004956829
10	PFOS	3.7	1.943437	2.614328	2.015931	10	PFOS	3.7	1.32766776	0.1288026	16.8033495		0.2118	0.924825023
11	PFOS	11.1	2.207644	2.396115	2.389978	11	PFOS	11.1	1.41250206	0.03746052	4.59351654		0.0839	0.789968427
12	PFOS	33.3	2.357472	2.187248	2.338161	12	PFOS	33.3	1.39011284	0.03260492	4.06250254		0.1081	0.518650202
13	PFOS	100	3.579363	3.781329	2.666823	13	PFOS	100	2.02522423	0.20772368	17.7653397		0.0001	0.610922973
14	PFOS217	0	1.912326	1.875983	1.163002	14	PFOS217	0	1	0.14780522	25.6006153	0.0021	1	1
15	PFOS217	0.41	1.317282	2.546865	1.013073	15	PFOS217	0.41	0.98503609	0.28408237	49.9519863		1	1.005491244
16	PFOS217	1.23	1.540737	1.696937	1.672663	16	PFOS217	1.23	0.99172462	0.02940407	5.135432		1	1.066335615
17	PFOS217	3.7	1.812527	2.455303	1.985599	17	PFOS217	3.7	1.26298449	0.11634902	15.9560482		0.91	0.947387028
18	PFOS217	11.1	1.414215	2.01943	1.870738	18	PFOS217	11.1	1.07130879	0.11032706	17.8372535		0.9999	0.827814675
19	PFOS217	33.3	3.215519	2.594508	2.204319	19	PFOS217	33.3	1.61863111	0.17839661	19.0897107		0.2754	0.650976949
20	PFOS217	100	3.44258	3.481387	5.685531	20	PFOS217	100	2.54669884	0.44913387	30.5463162		0.0014	0.636128925
21	PFHxS	0	1.810927	1.347109	1.06981	21	PFHxS	0	1	0.15340346	26.5702592	0.0516	1	1
22	PFHxS	0.41	1.250442	1.364983	1.598152	22	PFHxS	0.41	0.996625	0.07259294	12.6160452		1	0.98896434
23	PFHxS	1.23	1.558483	1.117709	1.35386	23	PFHxS	1.23	0.95321637	0.09036442	16.4197521		1	0.990124327
24	PFHxS	3.7	0.65403	1.186113	1.338007	24	PFHxS	3.7	0.75171849	0.14714271	33.9034695		0.9467	0.991382842
25	PFHxS	11.1	2.704604	1.69808	1.317618	25	PFHxS	11.1	1.35300624	0.29359727	37.5848512		0.8067	1.025955982
26	PFHxS	33.3	1.915768	2.378524	1.878478	26	PFHxS	33.3	1.46002716	0.11412028	13.5382502		0.6075	0.799988824
27	PFHxS	100	2.305565	1.811519	4.181846	27	PFHxS	100	1.96292155	0.51231352	45.2057319		0.064	0.541800852
28	PFBS	0	1.079713	1.296352	1.287055	28	PFBS	0	1	0.05791329	10.0308766	0.0525	1	1
29	PFBS	0.41	1.028022	1.083755	0.9279157	29	PFBS	0.41	0.82980975	0.03733765	7.79343841		0.8134	0.838473597
30	PFBS	1.23	0.280539	0.5447473	1.172045	30	PFBS	1.23	0.54525413	0.21651598	68.7783281		0.075	0.660562222
31	PFBS	3.7	1.663123	1.508355	0.978928	31	PFBS	3.7	1.13302485	0.16964702	25.9338761		0.922	0.830330148
32	PFBS	11.1	1.293376	1.477396	1.23914	32	PFBS	11.1	1.09467121	0.05904785	9.34288534		0.9822	0.925845375
33	PFBS	33.3	1.251064	1.159395	1.332643	33	PFBS	33.3	1.02183439	0.040982	6.9466146		1	0.850571568
34	PFBS	100	0.8910954	1.369629	1.274268	34	PFBS	100	0.96502228	0.11976036	21.4949473		0.9999	0.69897459
35	PFOA	0	1.089725	1.238347	1.168205	35	PFOA	0	1	0.03683287	6.37964081	0.0275	1	1
36	PFOA	0.41	1.243024	1.567674	1.695682	36	PFOA	0.41	1.28890817	0.11559589	15.5339193		0.5002	1.112374818
37	PFOA	1.23	1.937257	1.951602	0.9804638	37	PFOA	1.23	1.39271654	0.27573495	34.2917544		0.2323	1.245113855
38	PFOA	3.7	2.095318	1.740867	2.189428	38	PFOA	3.7	1.72343696	0.11718022	11.7765893		0.0104	1.122166898
39	PFOA	11.1	2.081964	1.814638	1.857409	39	PFOA	11.1	1.64575376	0.07113687	7.48670126		0.0224	1.156368033
40	PFOA	33.3	1.414356	1.651225	1.860067	40	PFOA	33.3	1.40882659	0.11047504	13.5821104		0.2032	0.981255019
41	PFOA	100	1.591633	1.223631	1.308074	41	PFOA	100	1.17935106	0.09549803	14.0252924		0.8577	0.675082953
42	PFBA	0	1.811601	2.122233	2.217719	42	PFBA	0	1	0.05978845	10.3556633	0.128	1	1
43	PFBA	0.41	2.717717	1.987632	1.445104	43	PFBA	0.41	0.99982118	0.17980789	31.1492104		1	1.017091648
44	PFBA	1.23	2.286602	2.237881	2.522597	44	PFBA	1.23	1.14557739	0.04287573	6.48257753		0.6792	1.148048428
45	PFBA	3.7	1.951135	2.10414	2.45519	45	PFBA	3.7	1.05834494	0.07276453	11.9083911		0.9909	1.023359028
46	PFBA	11.1	1.444449	1.721626	1.657182	46	PFBA	11.1	0.78407144	0.04084049	9.02185598		0.3261	1.008818678
47	PFBA	33.3	1.750552	1.918734	1.753327	47	PFBA	33.3	0.88150309	0.02711702	5.32817916		0.8218	1.122079641
48	PFBA	100	2.031571	2.429702	2.009645	48	PFBA	100	1.05191616	0.06657416	10.9618831		0.995	1.094378002

[1] Dose in nM for TO901317 and uM for client's compounds

[2] Expressed as (Luciferase RLU/Renilla RLU)

[3] Expressed relative to DMSO control

[4] Oneway Anova, Dose x Relative Luciferase, Prob > F

[5] Dunnett's t-test, DMSO control versus each sample

[6] Relative Renilla activity (toxicity marker)

Relative renilla below 50% (0.5) indicates overt toxicity

Curve Fitting Data

	CITCO	PFOS	PFOS217	PFHxS	PFBS	PFOA	PFBA
Bottom	0.7363	0.9705	1.009	0.9236	0.8209	Ambiguous	1.054
Top	42.9	2.328	10.65	2.601	1.053		0.9223
LogEC50	2.809	1.581	2.721	1.795	0.5115		0.5894
EC50	644.4	38.11	525.9	62.41	3.247		3.885
Span	42.16	1.357	9.645	1.677	0.2322		-0.1318

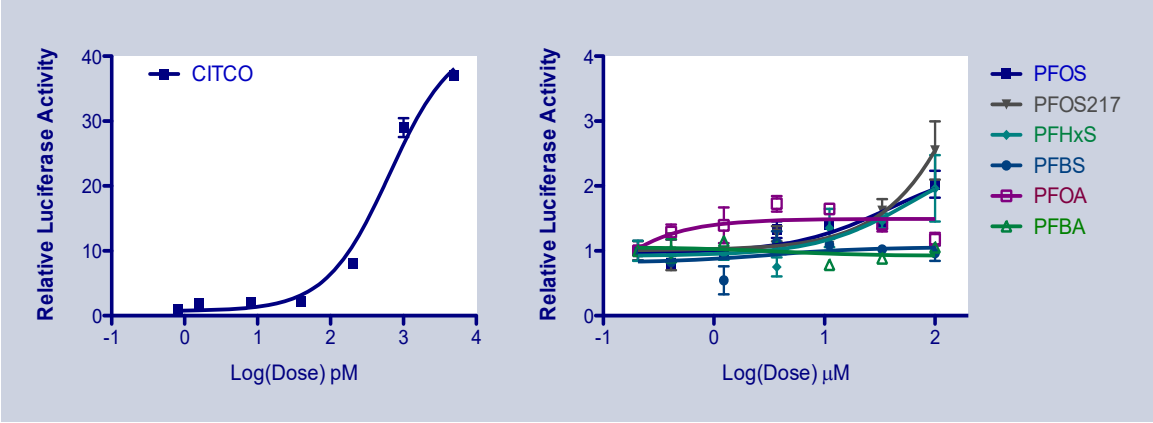
Statistical Analysis

	PFOS	PFOS217	PFHxS	PFBS	PFOA	PFBA
P-value	0.0001	0.0021	0.0516	0.0525	0.0275	0.128
Peak[4]	2.025	2.547	1.9629215	0.5452541	1.723437	0.7840714
LSSD[5]	100	100	N/A	N/A	3.7	N/A

[4] Highest observable response (relative to DMSO control)

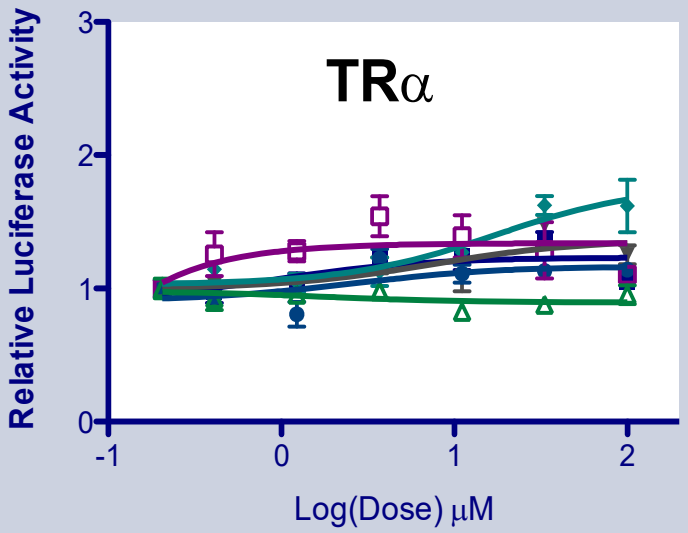
[5] Lowest statistically significant dose (p<0.05, Dunnett's)

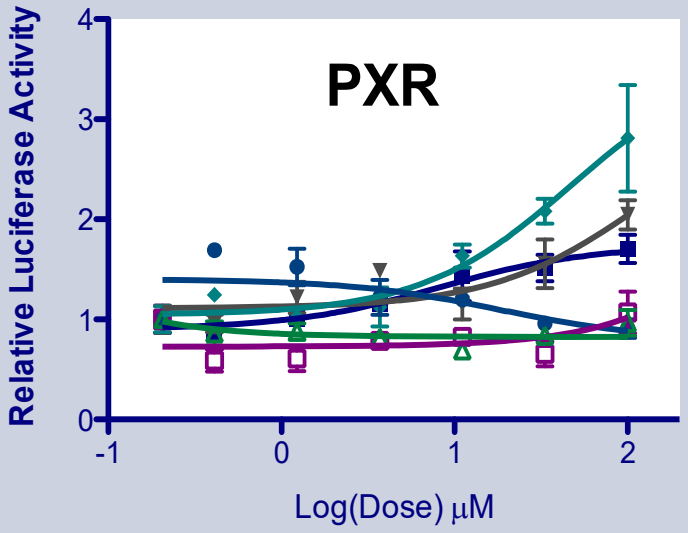
NA, not applicable

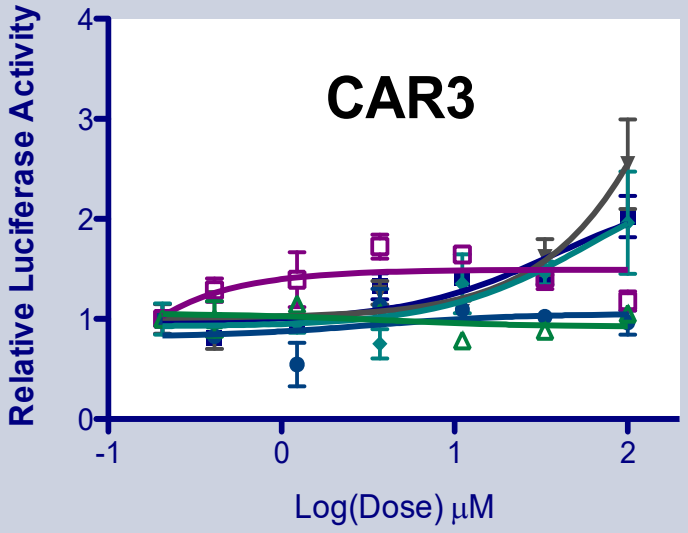


Statistical Summary:

	Statistical Analysis						
TRalpha		**PFOS**	**PFOS217**	**PFHxS**	**PFBS**	**PFOA**	**PFBA**
	P-value	0.0087	0.0014	0.0015	0.0035	0.1817	0.3298
	Peak[1]	1.360	1.475	1.6225666	1.2237863	1.5418959	0.8240689
	LSSD[2]	33.3	33.3	33.3	N/A	N/A	N/A
PXR	**Statistical Analysis**						
		PFOS	**PFOS217**	**PFHxS**	**PFBS**	**PFOA**	**PFBA**
	P-value	0.0032	0.0018	0.0005	0.0008	0.0765	0.031409
	Peak[1]	1.706	2.045	2.8101178	1.692992	1.0697926	0.6893013
	LSSD[2]	100	100	33.3	0.41	N/A	N/A
CAR3	**Statistical Analysis**						
		PFOS	**PFOS217**	**PFHxS**	**PFBS**	**PFOA**	**PFBA**
	P-value	0.0001	0.0021	0.0516	0.0525	0.0275	0.128
	Peak[1]	2.025	2.547	1.9629215	0.5452541	1.723437	0.7840714
	LSSD[2]	100	100	N/A	N/A	3.7	N/A


TRα
Relative Luciferase Activity
Log(Dose) μM
PFOS
PFOS217
PFHxS
PFBS
PFOA
PFBA


PXR
Relative Luciferase Activity
Log(Dose) μM
PFOS
PFOS217
PFHxS
PFBS
PFOA
PFBA


CAR3
Relative Luciferase Activity
Log(Dose) μM
PFOS
PFOS217
PFHxS
PFBS
PFOA
PFBA