

Supporting Information:

The use of pharmaceutical physiochemical properties to model their uptake by a fish primary gill cell culture epithelium

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The Supporting Information includes information on the cytotoxicity of the pharmaceuticals in primary gill cells (MTT assay), *in vivo* BCF data from literature, the HPLC method performance assessment, linear regression analysis of uptake rates in relation to the chemical descriptors and modelling supplementary information includes the scores, loadings, modified weights and cross-validation for PCA and PLS; 14 pages, 4 figures, 9 tables.

Supporting Information

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Table S9: Descriptor collinearity assessment using Pearson's correlation coefficient

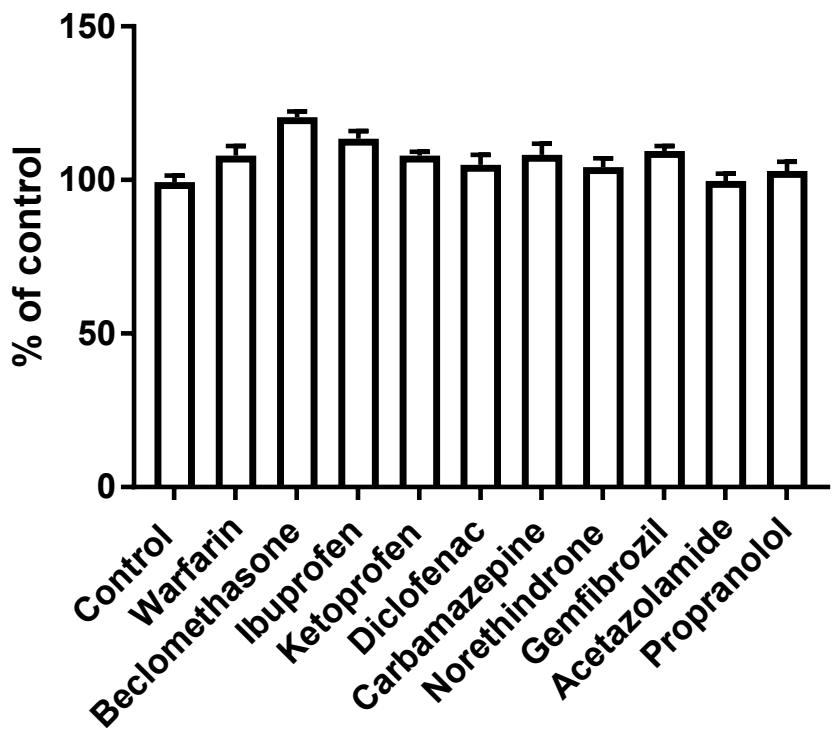


Figure S1. Pharmaceutical toxicity following 24 hours of exposure of primary gill cell culture to 1 μ g/ml as measured by MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

Table S1. HPLC instrument and method linearity.

Compounds	Retention time (R_t)	Instrument linearity	Method Linearity (0.5-2 μ g mL $^{-1}$)	LOD	LOQ	Recovery (RSD) (%)		
	R_t (SD)	R^2	R^2	ng mL $^{-1}$	ng mL $^{-1}$	0.5 μ g mL $^{-1}$	1 μ g mL $^{-1}$	2 μ g mL $^{-1}$
Warfarin	11.3 (0.001)	0.999	0.997	46.2	153.9	116 (17)	110 (9)	108 (4)
Ibuprofen	12.3 (0.016)	0.999	0.999	51.6	172.1	115 (7)	122 (14)	114 (6)
	14.4 (0.008)							
Gemfibrozil	15.7 (0.035)	0.999	0.995	58.4	194.6	80 (12)	97 (5)	120 (1)
Diclofenac	12.1 (0.11)	0.999	0.995	48.9	163.1	82 (13)	95 (6)	118 (2)
Ketoprofen	10.9 (0.014)	0.999	0.996	56.7	189.0	81 (18)	94 (7)	111 (2)
Norethindrone	15.8 (0.001)	0.999	0.973	13.4	44.6	96 (17)	120 (10)	112 (18)
Beclomethasone	14.7 (0.008)	0.995	0.963	108.3	361.0	104 (8)	109 (18)	108 (4)
Carbamazepine	14.2 (0.002)	0.996	0.998	6.9	23.0	84 (15)	97 (6)	108 (4)

Table S2. HPLC performance, intra- and interday reproducibility.

Compounds	Precision (RSD%)			Intraday repeatability (RSD%)			Interday reproducibility (RSD%)		
	0.5 µg mL ⁻¹	1 µg mL ⁻¹	2 µg mL ⁻¹	0.5 µg mL ⁻¹	1 µg mL ⁻¹	2 µg mL ⁻¹	0.5 µg mL ⁻¹	1 µg mL ⁻¹	2 µg mL ⁻¹
Warfarin	9.2	7.5	6.8	4.7	8.8	7.1	8.8	8.4	6.7
Ibuprofen	15.7	16.4	6.2	2.0	14.0	6.1	14.2	13.5	5.9
Gemfibrozil	12.5	12.5	15.9	7.5	4.8	4.4	11.9	17.3	12.9
Diclofenac	15.3	11.9	16.7	1.0	6.2	2.1	13.1	10.6	13.3
Ketoprofen	13.8	9.6	16.2	0.6	7.2	1.5	11.6	8.6	12.9
Norethindrone	19.2	13.9	16.0	12.0	10.4	18.3	16.2	12.5	16.6
Beclomethasone	17.8	17.5	17.7	17.0	18.2	13.8	14.0	13.5	5.9
Carbamazepine	11.1	6.3	11.4	2.7	6.0	0.3	9.4	5.6	9.6

Table S3: Pharmaceutical uptake rate across the fish gill cell culture system.

Compound	Rate (nmoles cm⁻² hour⁻¹)	SEM
Acetazolamide	0.125013	0.031925
Beclomethasone	0.02047	0.01504
Carbamazepine	0.021583	0.004528
Diclofenac	0.026767	0.003236
Gemfibrozil	0.079864	0.007231
Ibuprofen	0.071835	0.01279
Ketoprofen	0.06103	0.006528
Norethindrone	0.024397	0.003452
Propranolol	0.09507	0.025883
Warfarin	0.070062	0.011612

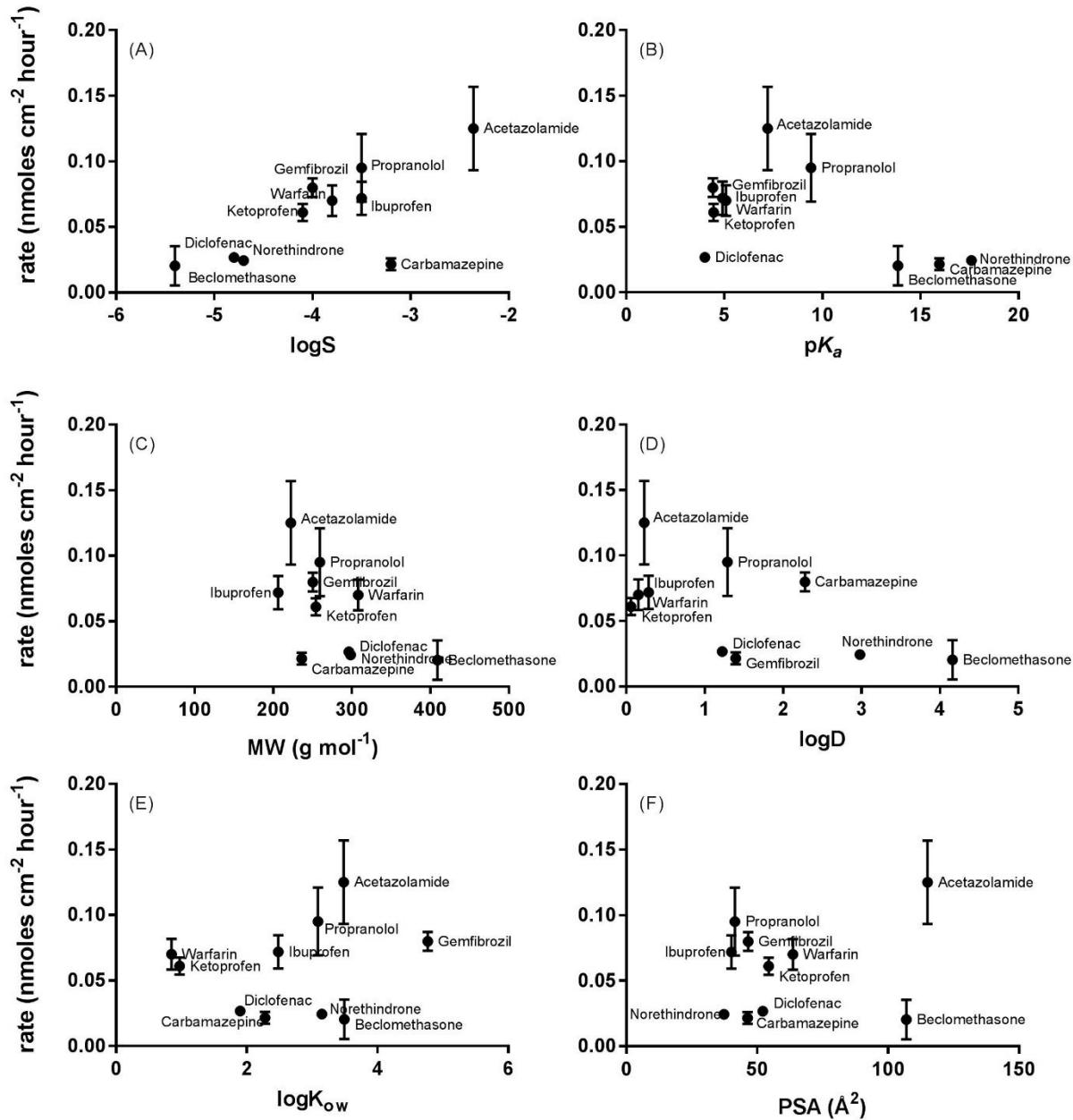


Figure S2: Linear regression analysis was used to identify the relationships between uptake and the various chemical parameters (molecular weight (MW), polar surface area (PSA), distribution constant coefficient ($\log D$), octanol water partition coefficient ($\log K_{ow}$), solubility ($\log S$), and pK_a and uptake rate following 24-hour exposure in FIGCS using GraphPad 6.0 Prism. Relationships were as follows: $\log S$, $R=0.74$, $p=0.02$, pK_a $R = 0.53$, $p = 0.12$, molecular weight, $R = 0.55$, $p = 0.1$; $\log D$, $R=0.57$, $p = 0.09$; $\log K_{ow}$, $R=0.2$, $p = 0.58$ and PSA, $R=0.25$ and $p = 0.5$.

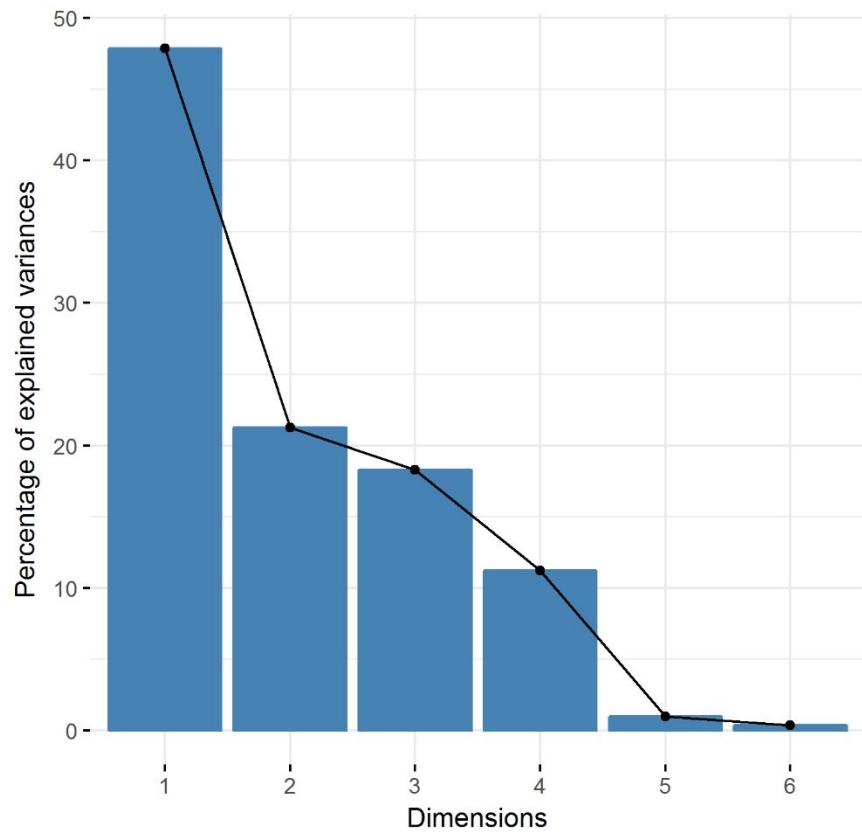


Figure S3: Scree plot of PCA modelling indicating the explained variance from each latent variable.

Table S4: Explained variance of latent variables in PCA model

	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	1.6947	1.1295	1.0479	0.8207	0.24138	0.15029
Proportion of Variance	0.4787	0.2126	0.183	0.1123	0.00971	0.00376
Cumulative Proportion	0.4787	0.6913	0.8743	0.9865	0.99624	1

Table S5: Scores of PCA model

	PC1	PC2	PC3	PC4	PC5	PC6
Warfarin	-0.85079	1.469985	0.691167	0.584021	0.448307	-0.04357
Ibuprofen	-1.70097	-0.01837	-0.54258	-0.34789	-0.19798	0.042981
Beclomethasone	3.838893	0.110819	1.314671	-0.05427	-0.03953	0.08737
Gemfibrozil	-0.229	-0.93022	-0.2776	-1.78083	0.107045	0.029887
Diclofenac	0.021939	1.406396	0.122817	-0.60137	-0.18493	0.11393
Ketoprofen	-1.28596	1.384867	0.158539	0.248955	-0.27851	-0.09058
Norethindrone	1.902831	-0.23556	-1.46421	0.295873	-0.07271	-0.30454
Carbamazepine	0.090676	-0.84291	-1.16296	1.291927	-0.05341	0.256637
Acetazolamide	-1.4226	-1.8908	1.82404	0.480732	-0.10327	-0.10505
Propranolol	-0.36501	-0.4542	-0.66389	-0.11714	0.374977	0.012947

Table S6: Loadings of PCA model

	PC1	PC2	PC3	PC4	PC5	PC6
pK _a	0.40684	-0.31423	-0.35568	0.614737	-0.03304	-0.47984
logS	-0.44355	-0.49347	0.076853	0.381939	0.539263	0.34229
logD	0.566621	-0.15601	-0.16682	0.041751	-0.17226	0.771592
logK _{ow}	0.20122	-0.68362	-0.00624	-0.65072	0.141672	-0.2205
MW	0.504726	0.334746	0.291989	-0.01276	0.736335	-0.07475
PSA	0.146439	-0.23241	0.868598	0.225562	-0.34084	-0.05504

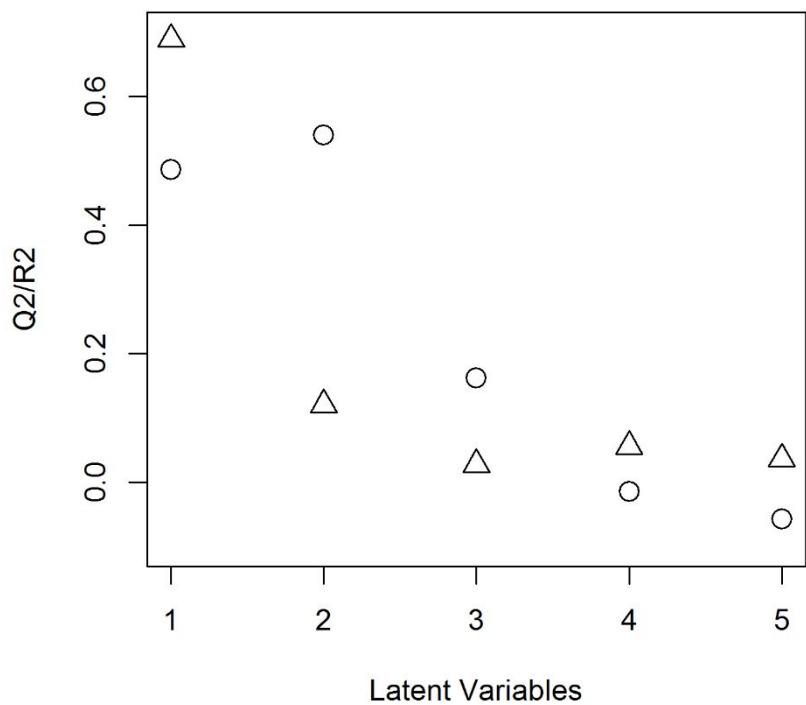


Figure S4: R^2 (triangles) and cumulative Q^2 (circles) for each additional latent variable used in the PLS regression model

Table S7: Modified weights of undeflated vectors to calculate PLS scores

	w*1	w*2
pK _a	-0.40418	-0.06206
logS	0.567583	0.247217
logD	-0.52421	-0.00026
logK _{ow}	0.153933	0.744307
MW	-0.42475	0.233071
PSA	0.188649	0.647016

Table S8 Internal pharmaceutical concentrations from *in vivo* experiments and predicted K₁, LC₅₀ and BCF values

Compound	Exposure conditions	Plasma concentration ng/mL	Ref	K ₁	LC ₅₀	BCF
				(L Kg ⁻¹ d ⁻¹)	(mg L ⁻¹)	(L Kg ⁻¹)
Acetazolamide	N/A	N/A	N/A	6.402	8170	0.90
Beclomethasone	pH 7.4, 21 day exposure, fathead minnow. Note the dipropionate is rapidly metabolized and this study reports the combined values	60 ± 35 ng/mL at 21 days exposure to 816 ng/L	Margiotta-Casaluci et al. 2016	6.420	318	8.95
Carbamazepine	pH 7.7 10 days, rainbow trout	25 ng/mL after 10 days exposed to 43 µg/L	Lahti et al 2011	4.244	40.9	19.30
Diclofenac	pH 7.6, 48h and 21 day, fathead minnow	205 ng/mL after 48h exposure to 24 µg/L. 26 ng/mL after 21 days exposed to 0.95 µg/L	Bickley et al 2017	3.550	37.7	728.60
Gemfibrozil	pH 7.6, 6h exposure, zebrafish	183 ng/mL after 6h exposure to 12.5 µg/L	Chen et al 2017	8.667	6.73	905.70
Ibuprofen	pH 7.4, fathead minnow 24h (but time course in paper)	550 ng/mL after 24h exposed to 107 µg/L	Patel et al 2016	4.607	41.6	223.90
Ketoprofen	pH 7.4, rainbow trout 14 day exposure	1 ng/mL after 14 days exposure to 91 µg/L	Cuklev et al 2012	1.829	264	95.91
Norethindrone	pH 7.4, 7 days	950 ng/mL exposed to 83 µg/L for 7 days	Nallani et al 2012	5.813	53.5	74.34
Propranolol	pH 7.4, 28 days, rainbow trout	0.94 ng/mL after 28 days exposed to 0.92 µg/L	Owen et al 2009	5.706	3270	1.38
Warfarin	N/A	N/A	N/A	1.604	30.2	20.00

K₁ calculated from Arnot and Gobas, 2003: A Generic QSAR for Assessing the Bioaccumulation Potential of Organic Chemicals in Aquatic Food Webs. Linear regression analysis of K1 and *in vitro* pharmaceutical uptake gave r²=0.039, p= 0.585.

LC₅₀ predicted using ECOSAR application 2.0. Linear regression analysis of LC₅₀ and *in vitro* pharmaceutical uptake gave r²=0.559, p=0.013.

BCF predicted using BCFBAF application in EpiSuite. Linear regression analysis of LC₅₀ and *in vitro* pharmaceutical uptake gave r²=0.003, p=0.875.

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Table S9: Descriptor collinearity assessment using Pearson's correlation coefficient

	pKa	logS	logD	logKow	Mw	PSA
pKa	1.00					
logS	-0.20	1.00				
logD	0.80	-0.63	1.00			
logKow	0.24	0.01	0.44	1.00		
Mw	0.34	-0.81	0.69	0.01	1.00	
PSA	0.02	0.08	0.13	0.18	0.38	1.00