Supplemental Information for :

Modeling oral up-take of hydrophobic and super-hydrophobic chemicals in fish

Content

Part I - GIT Surface Area

For a size specific scaling of GIT surface area including the ceca and villi structures, we need information on the amount and surface areas of ceca and the villi/ micro-villi in combination with fish size or weight. To this end , we used data from *Lee and Cossins 1988* [1] , where data on GIT mucosal surface area (including villi and microvilli) as a function of temperature is provided. They show that surface area per GIT length changes along the GIT tract (*Fig1*).

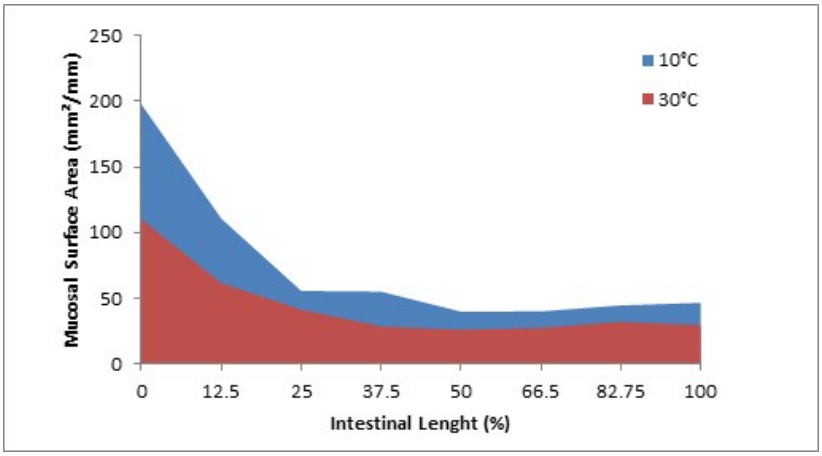

Figure 1 : Mucosal Surface Area versus intestinal length for two different temperatures. Data extracted from Lee and Cossins[1]

It is to be noted that the results from *Lee and Cossins* are for carp while we were interested in rainbow trout. *Buddington and Diamond* [2] show in their paper data for body-weight to GIT surface area for different species (without accounting for villi and microvilli) (*Fig2*) with only minor differences between carp and trout. So, we assume them to be equal for our purposes. *Lee and Cossins* 1988 provide data for 10°C and 30°C. To get a linear

relationship between GIT-length and surface area, we integrated the data from *Fig1*. After integration we normalized to mm² surface area per mm GIT length.

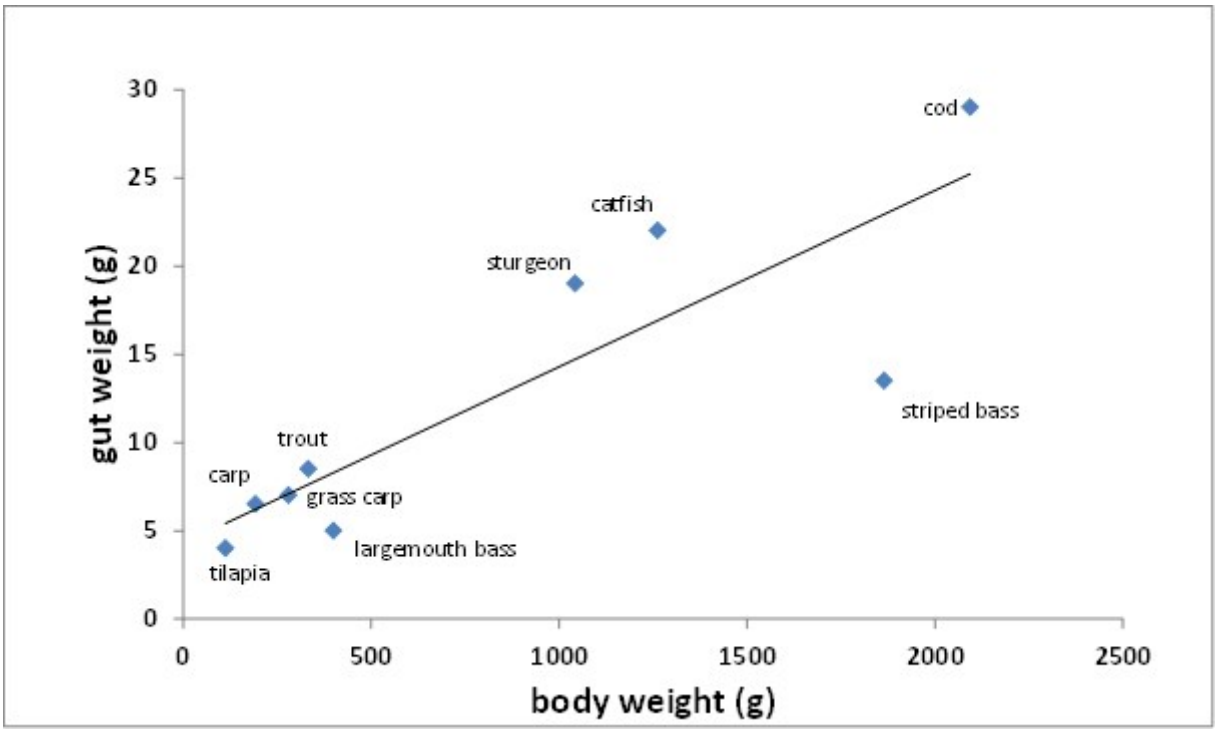

Figure 2: body-weight to gut-weight for different species from Buddington and Diamond 1987

We received a relationship of 63.75 mm²/ mm for 10°C and 39.46 mm² / mm for 30°C and used the average of 51.6 mm² / mm for 20°C. *Buddigton and Diamond* reported a bodyweight to GIT length relationship of 3.09 mm / g for trout. Combining this information leads to the final result of 1.6 cm²/g (surface area/ fish weight), which is roughly 7.5 times higher than the value used by *Buddigton and Diamond* without accounting for villi and microvilli.

Part II - Bile Water Partition Coefficient

To estimate a bile/water partition coefficient (K_{bw}), available sorption data for bile/PDMS from Zhang et al 2015 were used [3]. With PDMS / water partition coefficients from the UFZ LSER database [4], bile/water partition coefficients could be calculated via a thermodynamic cycle. The list of substances from Zhang and the corresponding partition coefficients can be seen in *Table1* .

Table 1 : Partition Coefficients of different substances from Zhang[3] et al 2015 and the UFZ LSER Database[4]

PAH'S	Log $K_{PDMS/bile}$	Log $K_{PDMS/Water}$	Log $K_{bile/water}$	Log K_{ow}
Fluorene	2.34	3.63	1.29	4.16
Phenantrene	2.2	3.73	1.53	5
Pluranthen	2.15	4.16	2.01	5.06
Pyrene	2.08	3.76	1.68	4.99
Benzo(a)anthracene	2.11	4.95	2.84	5.64
Chrysene	2.04	4.66	2.62	5.76
Benzo(b)fluoranthene	1.94	5.04	3.1	5.78
Benzo(k)fluoranthene	1.98	5.16	3.18	6.39
benzo(a)pyrene	1.87	4.67	2.8	6.45
Indeno(1,2,3-cd)pyrene	1.91	5.49	3.58	7.53
Benzo[ghi]perylene	1.77	5.63	3.86	6.8

A simple log Kow relationship was derived when the bile partition data were plotted against the log Kow (see *Fig3*). The derived relationship is $log\ K_{bw} = 0.812 * log\ K_{ow} - 2.10$

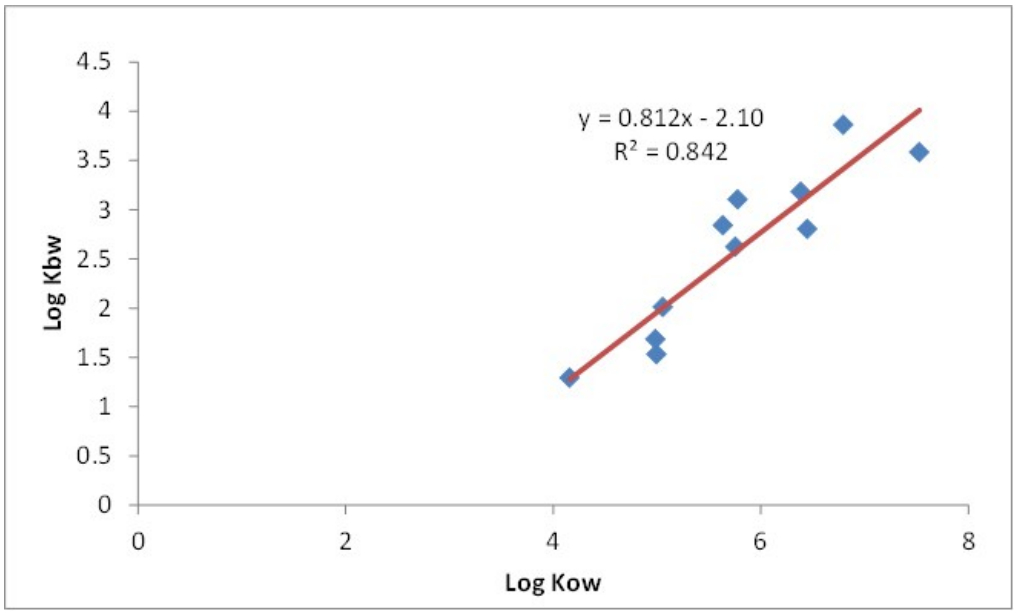

Figure 3: Ploted Sorption data from Zhang et al 2015.

Part III - Permeability with Facilitation Factor

Facilitation through Blood-Albumin

For estimating a facilitation factor we consider two transport processes through the aqueous boundary layer (ABL) that occur in parallel. One is passive diffusion of the freely dissolved fraction of the substance and the other is the carrier bound fraction that is transported while the carrier diffuses through the ABL. Because permeability (P) for parallel processes is additive, we can write the following equation for the facilitation factor which is defined as the quotient of the combined processes over the single, only passive diffusion process:

$$Facilitation = \frac{P_{passive\ diffusion}^{ABL} + P_{carrier\ bound}^{ABL}}{P_{passive\ diffusion}^{ABL}}$$

The carrier based permeability can be calculated as follows:

$$P_{carrier\ bound}^{ABL} = \frac{D_{carrier\ in\ water} * K_{carrier\ /water} * F_{carrier\ inwater}}{s_{ABL}}$$

Where $D_{carrier/water}$ is the diffusion coefficient of the carrier in water, $K_{carrier/water}$ is the partition coefficient of the transported chemical between the carrier and water, F the volume fraction of the carrier in water and s_{ABL} is the thickness of the ABL.

The passive permeability of the substance itself is calculated in an equivalent approach:

$$P_{passive\ diffusion}^{ABL} = \frac{D_{in\ water}}{s_{ABL}}$$

Where $D_{in\ water}$ is the diffusion coefficient of the substance in water.

Aa an example we show the data for the simulation of hexachlorobenzene in a 1.5 g rainbow trout from the OECD ring test 2011 (see Table2), where the calculated facilitation factor is 6.38 . The albumin / water partition coefficients were calculated using the UFZ-LSER database[4].

Table 2 : Variables for calculating the facilitation factor for hexachlorobenzene in rainbow trout. All variables are refering to 20°C.

Variable	Value	Unit
s_{ABL}	0.286	µm
$K_{albumin\ /water}$	4.30 E+03	L_{water} / $kg_{albumin}$
$F_{albumin\ in\ water}$	0.02054	Volume Fraction
$D_{in\ water}$	0.59 E-5	cm²/sec
$D_{albumin\ in\ water}$	3.6 E-7	cm²/sec
$P_{passive\ diffusion}$	0.2065	cm /sec
$P_{carrier\ based}$	1.112	cm /sec

Micelle facilitation in the GIT

In order to estimate a micelle facilitation in the GIT we used data from Westergaard and Dietschy 1976 [5]. The authors measured the uptake kinetics of fatty acids of different chain length in the presence and absence of a micelle building bile acid (Taurodeoxycholate). They found that the influence of the bile acid increases with decreasing solubility of the fatty acids (see Fig4).

TABLE I

Effect of Bile Acid Micelles on Increasing the Maximum Rates of Fatty Acid and Cholesterol Uptake into the Intestinal Mucosal Cell

(A) Probe molecule	(B) Maximum solubility in bulk Krebs' buffer, C_1	(C) Passive permeability coefficient, P	(D) $\frac{C_2}{C_1}$	(E) Maximum uptake, J_d, in absence of bile acid micelles	(F) Maximum uptake, J_d, in presence of bile acid micelle at microvillus interface	(G) $\frac{F}{E}$
	mM	*nmol·min⁻¹·100 mg⁻¹·mM⁻¹*		*nmol·min⁻¹ ·100 mg⁻¹*	*nmol·min⁻¹ ·100 mg⁻¹*	
FA 4:0	575	4.7	1.00	2,703	2,703	1.00
6:0	107	11.9	0.99	1,261	1,273	1.01
8:0	19.8	29.9	0.95	562	592	1.05
10:0	3.68	74.8	0.86	237	275	1.16
12:0	0.682	187	0.68	86.7	128	1.48
14:0	0.127	468	0.43	25.6	59.4	2.32
16:0	0.0235	1,170	~.00	7.78	27.5	3.53
18:0	0.00437	2,930	~.00	1.35	12.8	9.48
20:0	0.00081	7,330	~.00	0.23	5.9	25.83
22:0	0.00015	18,300	~.00	0.04	2.7	68.75
Cholesterol	0.00004	40,000	~.00	0.01	1.6	145.5

Figure 4: Tabel from Westergaard and Dietschy showing the results of the measured uptake in presence of a micelle building bile acid.

We used this relationship between sollubility and faciliation factor to estimate the facilitation effect for those compounds that we investigated. Before doing so we had to check whether the reported solubility values referred to subcooled liquids. Therefore we plotted the values of solubility against the number of C-atoms in the corresponding fatty acid (Fig5). All fatty acids from chain length 4:0 till 8:0 should be liquid and those above

should be solid at room temperature. The plot shows a straight line and no kink at 8 carbon atoms indicating that all data in the table represented (subcooled) liquid solubilities.

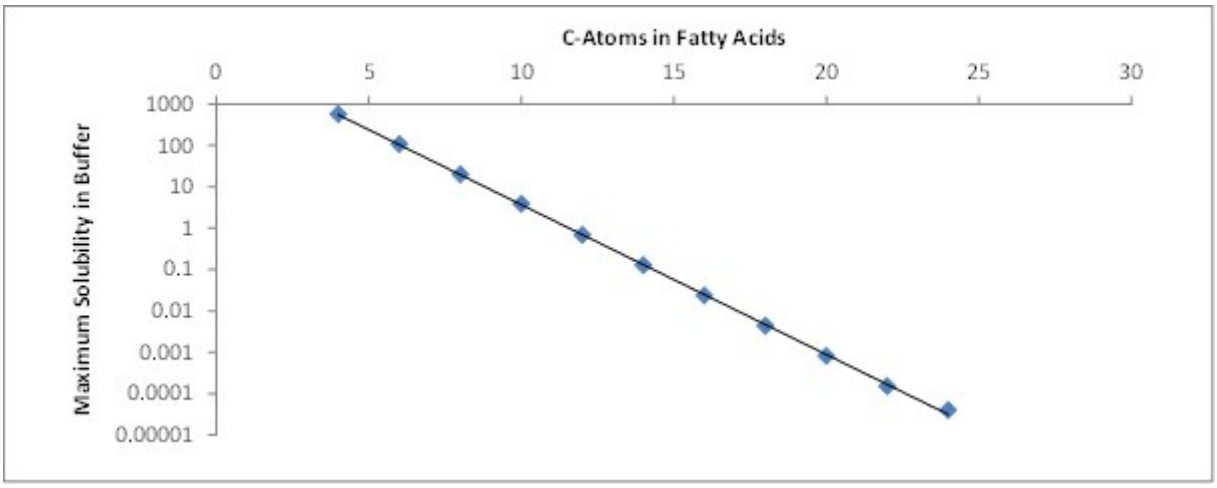

Figure 5: Solubility plotted against the number of c-atoms of the fatty acids from Westergaard and Dietschy 1976

For HCB we compared the solubility value from Schwarzenbach 1993 [6] (2.3*10^-3 mM) with the Table from Westergaard and Dietschy and derived a facilitation factor of 15.

Part IV – Model Data

All physiological data that are used for the 50g rainbow trout can be seen in the additional SI-Rainbowtrout_100.xlsx

All physiological data that are used for the 1.3g rainbow trout can be seen in the additional SI-Rainbowtrout_1_3.xlsx. Slight adaptions were made for the calculation for fish wet weight of 8.4g (Lab2) and 1.2g (Lab4).

Food compositions that were used to model the uptake behavior from the OECD Ring Test 2011 (No.175) can be seen in Table 3.

Table 3 : Experimental information from OECD Ring Test 2011 used in the PbTk model [7]. Fiber and Ash is considered as non-sorbing material. In the model, food composition is adjusted to 100% by the addition of water.

	Laboratory 1	**Laboratory 2a**	**Laboratory 4**
Temperature	14°C	15°C	14.5°C
Fish Wet Weight	1.3 g	8.4 g	1.2 g
Average Feeding	0.06 g	0.252 g	0.036 g
Food Composition:			
Protein	49.9%	55.0%	50%
Fat	10.3%	15.0%	16%
Fiber	1.6%	2%	1%
Ash	12.9%	---	10%
Concentration	47.2 mg/l	51.2 mg/l	47.9 mg/l

References

1 J. A. C. Lee and A. R. Cossins, *Cell Tissue Res.*, 1988, **251**, 451–456.

2 R. K. Buddington and J. M. Diamond, *Am. J. Physiol.*, 1987, **252**, G65-76.

3 Y. Zhang, J. J. Pignatello, S. Tao and B. Xing, *Env. Sci Technol*, 2015, 1–17.

4 K.-U. Endo, S., Watanabe, N., Ulrich, N., Bronner, G., Goss, *UFZ-LSER database v 2.1 [Internet], Leipzig, Deutschland, Helmholtz Zent. für Umweltforsch. - UFZ*, 2015.

5 H. Westergaard and J. M. Dietschy, *J. Clin. Invest.*, 1976, **58**, 97–108.

6 R. P. Schwarzenbach, P. M. Gschwend and D. M. Imboden, *Environmental Organic Chemistry*, John Wiley & Sons, NY, 1993.

7 *Validation report of a ring test for the OECD 305 dietary exposure bioaccumulation fish test, Organisation for Economic Co-operation and Development (OECD),www.oecd.org/chemicalsafety/testing/seriesontestingandassessmenttestingforenvironmentalfate.htm*, 2012, vol. 175.