Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

SUPPORTING INFORMATION

An amended *in vitro-in vivo* extrapolation model that accounts for first pass clearance effects on chemical bioaccumulation in fish.

Leslie J. Saunders[a], John W. Nichols[b], Jon A. Arnot[a,c],
James M. Armitage[d], Frank Wania[a]

[a]Department of Physical and Environmental Science, University of Toronto Scarborough, Toronto, Ontario, Canada
[b]United States Environmental Protection Agency, Duluth, MN, USA
[c]ARC Arnot Research and Consulting, Toronto, Ontario, Canada
[d]AES Armitage Environmental Sciences Inc., Ottawa, Ontario, Canada

6 pages containing 3 tables and 2 figures

Content Summary

TABLES

Table S1. Hepatic extraction ratios (*ER*; unitless) calculated at different assumed levels of *in vitro* hepatic activity ($k_{DEP,H}$; h^{-1}) at log K_{OW} between 4 and 10. The mean *ER* value at each $k_{DEP,H}$ between log K_{OW} 4 and 10 is also provided.

$k_{DEP,H}$	log K_{OW}							Mean
	4	5	6	7	8	9	10	
0.01	0.0008	0.0007	0.0006	0.0005	0.0005	0.0004	0.0004	0.0006
0.05	0.0042	0.0033	0.0029	0.0027	0.0024	0.0022	0.0021	0.0028
0.1	0.0083	0.0065	0.0058	0.0053	0.0049	0.0045	0.0041	0.0056
0.5	0.0403	0.0318	0.0283	0.0259	0.0239	0.0220	0.0203	0.0275
1	0.0775	0.0616	0.0551	0.0505	0.0466	0.0431	0.0398	0.0535
5	0.2957	0.2472	0.2256	0.2102	0.1965	0.1837	0.1716	0.2187
10	0.4564	0.3964	0.3682	0.3474	0.3285	0.3104	0.2930	0.3572
50	0.8076	0.7666	0.7445	0.7269	0.7098	0.6924	0.6745	0.7318

Table S2. Input parameters used for the extrapolation of *in vitro* biotransformation data [1] used to derive extraction ratios for the liver (ER_H) and intestinal epithelia (ER_G). Extraction ratios for the liver and intestinal epithelia are presented in Table 3 of the main text.

Parameter	BAP	PYR	EHMC	OCT	Source
Chemical properties					
Octanol-water partition coefficient (K_{OW}; L water L octanol^{-1})	6.09	4.88	5.80	6.88	[1]
Fish characteristics					
Weight of extrapolated fish ($W_{B,E}$; g)	10	10	39	36	[1]
Acclimation temperature (T; °C)	11	11	11	11	[1]
Well-stirred liver model inputs					
Fractional tissue blood flow (Q_{FRAC}; unitless)	0.259	0.259	0.259	0.259	[2]
Cardiac output (Q_C; L blood d^{-1} kg fish^{-1})	62	62	54	54	[3]
Clearance binding term (f_U; unitless)	0.022	0.026	0.023	0.021	[1]
Tissue blood flow (Q_L; L blood d^{-1} kg fish^{-1})	7.16	7.16	7.16	7.16	$Q_C \times Q_{FRAC}$
Water content of S9 (v_{WS9}; L water L S9^{-1})	0.84	0.84	0.84	0.84	[4]
Water content of blood (v_{WBL}; L water L blood^{-1})	0.99	0.99	0.99	0.99	[5]
Clearance estimates					
In vitro hepatic depletion rate constant ($k_{DEP,H}$; h^{-1})	39.90	17.98	3.60	0.88	[1]
In vitro intestinal epithelia depletion rate constant ($k_{DEP,G}$; h^{-1})	9.35	4.23	1.42	1.68	[1]
In vivo hepatic intrinsic clearance rate ($CL_{INT,LIV}$; L S9 d^{-1} kg fish^{-1})	1172	528	105	25	[1]
In vivo intestinal epithelia intrinsic clearance rate ($CL_{INT,GIT}$; L S9 d^{-1} kg fish^{-1})	417.6	170.0	60.7	65.7	[1]
Hepatic clearance rate (CL_H; L blood d^{-1} kg fish^{-1})	10.46	7.95	2.35	0.60	Table 2 of main text
Intestinal epithelia clearance rate (CL_G; L blood d^{-1} kg fish^{-1})	5.88	3.41	1.26	1.24	Table 2 of main text
Liver extraction ratio (ER_H; unitless)	0.650	0.488	0.141	0.043	CL_H/Q_L
Intestinal epithelia extraction ratio (ER_G; unitless)	0.366	0.244	0.092	0.097	CL_G/Q_L

Table S3. Oral bioavailability values (F), extraction ratios for the intestinal epithelia (ER_G) and liver (ER_H), and calculated luminal gut transfer efficiency values ($E_{D,LB}$; Equation 14 of main text) for test chemicals benzo(a)pyrene (BAP), pyrene (PYR), 2-ethylhexyl-4-methoxycinnamate (EHMC), and octocrylene (OCT). Values of ER_G, ER_H, and $E_{D,LB}$ were derived under different assumptions for *in vitro* activity (*in vitro* depletion rate constants in the liver [$k_{DEP,H}$] and the intestinal epithelia [$k_{DEP,G}$] are two and three-fold higher) and blood flows (blood flow rates [Q_L] calculated at a lower [0.1] and higher [0.4] fractions of cardiac output [Q_C]). All metrics are unitless values.[a]

Metric	BAP	PYR	EHMC	OCT
F	0.071[b]	0.020[c]	0.083[d]	0.031[d]
$2 \times k_{DEP,H}$ and $2 \times k_{DEP,G}$				
ER_H	0.785	0.654	0.247	0.083
ER_G	0.535	0.366	0.168	0.177
$E_{D,LB}$	0.710	0.091	0.132	0.041
$3 \times k_{DEP,H}$ and $3 \times k_{DEP,G}$				
ER_H	0.843	0.737	0.329	0.120
ER_G	0.633	0.464	0.232	0.244
$E_{D,LB}$	1.000	0.142	0.160	0.046
$Q_L = 0.1 \times Q_C$				
ER_H	0.824	0.709	0.297	0.105
ER_G	0.599	0.427	0.207	0.218
$E_{D,LB}$	1.000	0.120	0.148	0.044
$Q_L = 0.4 \times Q_C$				
ER_H	0.548	0.382	0.096	0.029
ER_G	0.272	0.157	0.061	0.065
$E_{D,LB}$	0.216	0.038	0.097	0.034

[a]Values of ER_G, and ER_H were derived from k_{DEP} values given by Saunders et al. [1] (Table S3)

[b]Average of values reported by Lo et al. [6,7] (n=3)

[c]Data obtained from Lo et al. [7] (n=1)

[d]Average of values reported by Saunders et al. [8] (n=3)

FIGURES

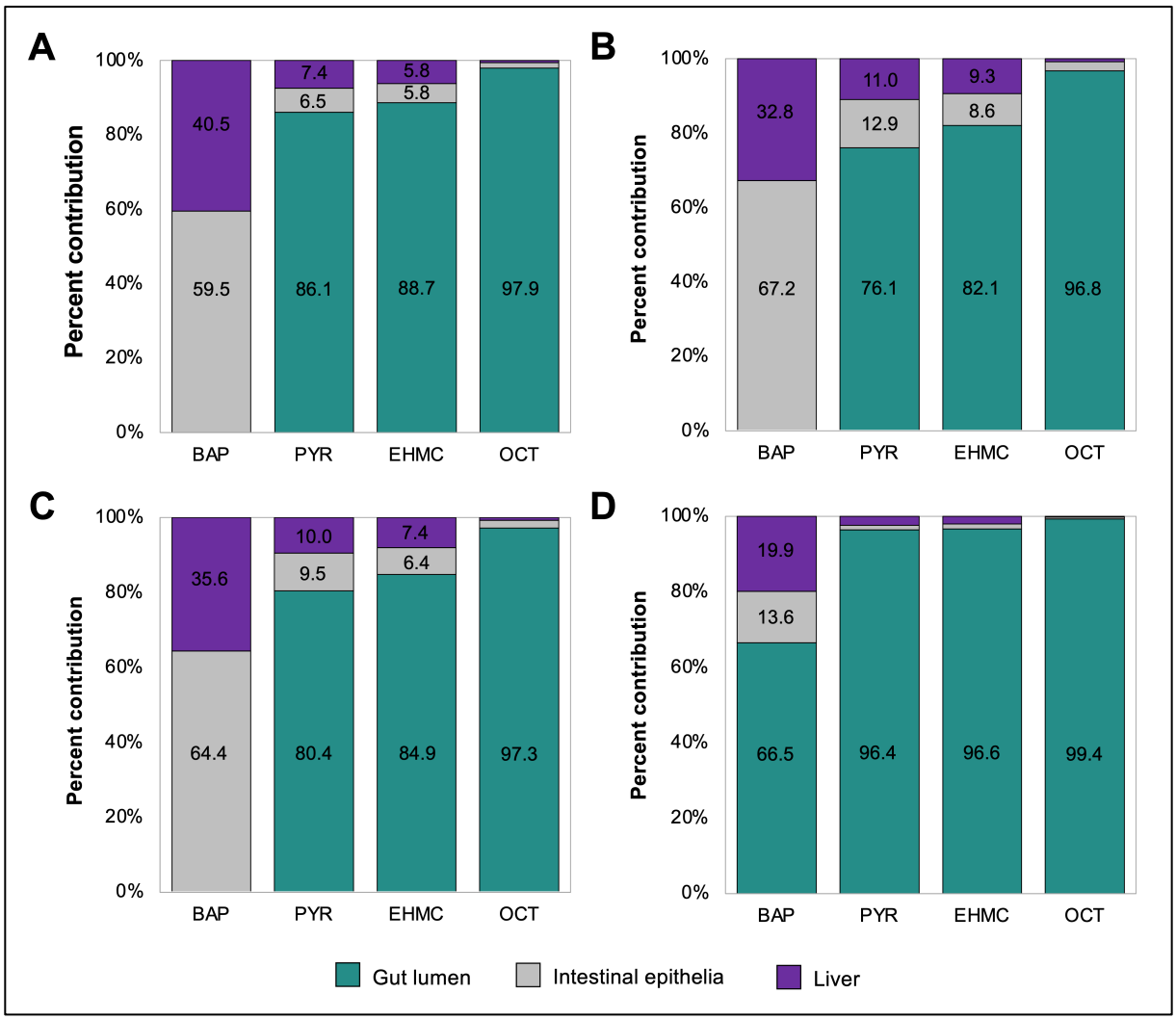

Figure S1. Estimated percent contribution of the liver (purple bars; Equation 15 of main text), intestinal epithelia (grey bars; Equation 16 of main text), and gut lumen (green bars; Equation 17 of main text) to overall presystemic biotransformation expressed relative to total contribution across all three compartments. Calculations are performed for the test chemicals benzo(a)pyrene (BAP), pyrene (PYR), 2-ethylhexyl-4-methoxycinnamate (EHMC), and octocrylene (OCT). The different panels display estimates using different estimates for *in vitro* activities in the liver ($k_{DEP,H}$) and intestinal epithelia ($k_{DEP,G}$) and for blood flow (Q_L): $2 \times k_{DEP,H}$ and $2 \times k_{DEP,G}$ (Panel A), $3 \times k_{DEP,H}$ and $3 \times k_{DEP,G}$ (Panel B), $Q_L = 0.10 \times Q_C$ (Panel C), $Q_L = 0.40 \times Q_C$ (Panel D) where Q_C is cardiac output. Fractional contributions were calculated from parameters given in Table S3.

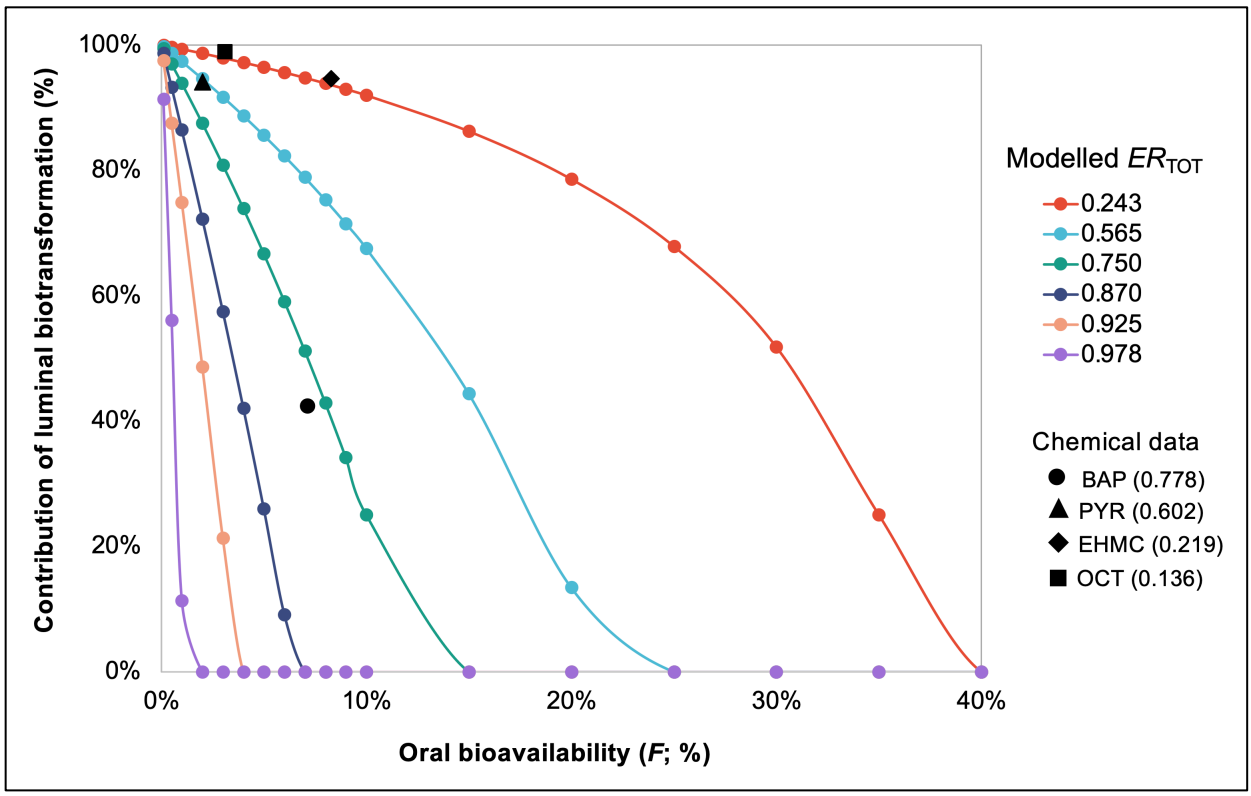

Figure S2. Estimated contribution of luminal biotransformation as a function of oral bioavailability (F). Each colored line represents a modelled total extraction ratio (ER_{TOT}) calculated from the individual extraction ratios for the liver (ER_H) and intestinal epithelia (ER_G) as $1-ER_{TOT}=(1-ER_H) \times (1-ER_G)$. The black datapoints represent the empirical data for four chemicals benzo(a)pyrene (BAP), pyrene (PYR), 2-ethylhexyl-4-methoxycinnamate (EHMC), and octocrylene (OCT). The empirical ER_{TOT} values are displayed in brackets in the figure legend and were generated for each chemical using input parameters described in Table S2.

REFERENCES

[1] Saunders LJ, Fitzsimmons PN, Nichols JW, Gobas FAPC. 2020. 'In vitro-in vivo extrapolation of hepatic and gastrointestinal biotransformation rates of hydrophobic chemicals in rainbow trout'. *Aquat Toxicol.* 228, 105629

[2] Nichols JW, Huggett DB, Arnot JA, Fitzsimmons PN, Cowan-Ellsberry CE. 2013. Towards improved models for predicting bioconcentration of well-metabolized compounds by rainbow trout using measured rates of in vitro intrinsic clearance. *Environ. Toxicol. Chem.* 32, 1611–1612.

[3] Erickson RJ, McKim JM, 1990. A model for exchange of organic chemicals at fish gills: Flow and diffusion limitations. *Aquat. Toxicol.* 18, 175–198.

[4] Bertelsen SL, Hoffman AD, Gallinat CA, Elonen CM, Nichols JW. 1998. Evaluation of logK_{OW} and tissue lipid content as predictors of chemical partitioning to fish tissues. *Environ. Toxicol. Chem*. 17, 1447–1455.

[5] Saunders LJ, Fontanay S, Nichols JW, Gobas FAPC. 2019. Concentration dependence of in vitro biotransformation rates of hydrophobic organic sunscreen agents in rainbow trout S9 fractions: Implications for bioaccumulation assessment. *Environ. Toxicol. Chem.* 38, 548–560.

[6] Lo et al. 2015 Lo, J.C., Campbell, D.A., Kennedy, C.J., Gobas, F.A.P.C., 2015. Somatic and gastrointestinal in vivo biotransformation rates of hydrophobic chemicals in fish. E*nviron. Toxicol. Chem.* 34, 2282–2294.

[7] Lo, J.C., Letinski, D.J., Parkerton, T.F., Campbell, D.A., Gobas, F.A.P.C., 2016. In vivo biotransformation rates of organic chemicals in fish: Relationship with bioconcentration and biomagnification factors. *Environ. Sci. Technol.* 50, 13299–13308.

[8] Saunders, L.J., Hoffman, A.D., Nichols, J.W., Gobas, F.A.P.C., 2020. Dietary bioaccumulation and biotransformation of hydrophobic organic sunscreen agents in rainbow trout. *Environ. Toxicol. Chem.* 39, 574–586.