Supporting Information (SI)

Biotransformation of benzo(a)pyrene by three rainbow trout (*Onchorhynchus mykiss*) cell lines and extrapolation to derive a fish bioconcentration factor

Julita Stadnicka-Michalak[1,2,§], Frederik T. Weiss[1,3,§], Melanie Fischer[1], Katrin Tanneberger[1,4], Kristin Schirmer[1,2,3*]

[1]Eawag, Überlandstrasse 133, 8600 Dübendorf, Switzerland
[2]EPF Lausanne, School of Architecture, Civil and Environmental Engineering, 1015 Lausanne, Switzerland
[3]ETH Zürich, Institute of Biogeochemistry and Pollutant Dynamics, 8092 Zürich, Switzerland
[4]Ecosens AG, 8304 Wallisellen, Switzerland

§ These authors contributed equally to this work

* Corresponding author:
Prof. Dr. Kristin Schirmer
Eawag, Swiss Federal Institute of Aquatic Science and Technology
Überlandstrasse 133
8600 Dübendorf
Switzerland
Phone: +41 (0)58 765 5266
Fax: +41 (0)44 823 53 11
email: kristin.schirmer@eawag.ch

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(1) Quantification of cell viability

For measuring cell viability after the exposure of cells in 48-well plates to BaP, cell viability was assessed exactly as previously described[1, 2] using a mixture of alamarBlue (Invitrogen, Switzerland), as a measure of the disturbance of metabolic activity, and 5-carboxyfluorescein diacetate acetoxy methyl ester (CFDA-AM; Invitrogen, Switzerland), as indicator of adverse effects to plasma membrane integrity. Fluorescence was quantified with the Infinite M200 multi-well plate reader (TECAN, Switzerland) at excitation and emission wavelengths of 530 and 595 nm for alamarBlue and 493 and 541 nm for CFDA-AM. The fluorescence units for the blank control (BaP only) were consistently subtracted from the results of the other wells but amounted to less than 8% of the negative and DMSO control values for alamarBlue and less than 1% for CFDA-AM. Negative controls and DMSO control values were comparable, confirming the lack of impact of this solvent on the assessed measures of cell viability.

(2) Chemical analysis: BaP extraction and quantification

Extraction of the medium: 1 mL of the exposure medium was added to 3.96 mL of ACN and 40 µL of acetic acid into a 7 mL amber glass vial. The acetic acid protonated the formed BaP-metabolites and helped to translocate the BaP metabolites from the H_2O-phase into the ACN-phase by reducing the pH of the extract to 2.

Extraction of the cells: The remaining exposure medium was carefully removed. 1 mL of an EDTA solution (Versene, 15040 Gibco; Invitrogen, Switzerland) was used to wash the cells after which 1 mL of trypsin (0.25 % in PBS w/o Ca^{2+}, Mg^{2+}; L0910, Biowest, France) was added to detach the cells. The cell-trypsin solution was added to 3.975 mL of ACN and 25 µL of acetic acid into a 7 mL amber glass vial.

Extraction of the plastic: After removal of the cells, 5 mL of ACN were added to the flasks and inner flask surfaces washed for several minutes by rigorous shaking to extract the BaP from the plastic walls. The 5 mL of ACN were transferred into a 7 mL amber glass vial.

To complete the extraction process, all the sample vials were placed into an ultra-sonic (VWR, Switzerland) bath for 10 minutes. The 7 mL vials were shaken by an IKA® Vortex Genius 3 for one hour to extract all the BaP into the ACN phase. Then, the extracts were frozen at -20°C, for at least two hours to precipitate proteins and lipids.[3] Finally, 200 µL of the supernatant (ACN phase only) were transferred into a 2 mL HPLC auto sampling vial with

a glass insert (Vials: BGB Analytik, Switzerland; screw cups with septum and glass inserts: Omnilab-VWR, Switzerland) for further analysis.

Each fraction was quantified via radio-HPLC, injecting 100 µL sample volume. A pre-column, and as stationary phase a C18 based HYPERSIL Green PAH column (150 * 2.1 mm particle size of 5 µm; Thermo Scientific, Switzerland), were used for analysis. All HPLC-analyses were performed using a flow rate of 500 µL/min of an ACN (1) and H_2O/0.2% acetic acid (v/v) (2) solvent mix, applying a partly isocratic and partly linear reversed phase gradient: 1-3 min → 60% (1), 40% (2); 3-6 min → 80% (1), 20% (2); 6-9 min → 100% (1), 0% (2); 9-11 min → 100% (1), 0% (2); 11-11.1 min → 60% (1), 40% (2); 11.1-17.3 min → 60% (1), 40% (2), maintaining column temperature at 40°C. For the radio-HPLC detection, the scintillation cocktail (Ultima FLOW^{TM}-M; PerkinElmer, Switzerland) was used for measuring the radio activity. The ratio between the scintillation cocktail and the HPLC solvent was 1:2. The minimal detectable activity was determined to be 38.95 nM. The determination of the background, the efficiency and the minimum detectable activity of the radio-HPLC detector was accomplished according to the reference manual of the Flow Scintillation Analyzer, Radiomatic^{TM} 500TR-Series. It should be noted that this method focused on rapid and sensitive determination of the kinetics of BaP; it does not allow to distinguish individual BaP metabolites.

For quantification of the parent ^{14}C-BaP, a calibration curve with BaP concentrations of 5, 20, 50, 200, 500, 1000, 2000 nM was prepared. The parent BaP had a retention time of about 11 minutes. The peak area of the radio-HPLC chromatograms were converted into decay per minute (DPM) and the amount of the parent BaP subsequently converted from DPM in nmol by considering the ratio between the labelled and the unlabelled BaP of the working solution. The limit of quantification (LOQ) was calculated based on the Minimal Detectable Activity (MDA), multiplied by a factor of 5 and amounted to 0.02 µmol/L for the plastic and the cell fraction and 0.2 µmol/L for the medium.

(3) PBTK Model:

3. a. Abbreviations and symbols used to describe the PBTK model

Table S-1. Abbreviations and symbols used to describe the PBTK model

Abbreviation /symbol	Units	Value	Description
K_{ow}	—	6.13 *(model input)*	octanol-water partition coefficient
C_{w_total}	$\mu g \cdot L^{-1}$	1 *(model input)*	Total aqueous chemical concentration
$C_{w,BaP}$	$\mu g \cdot L^{-1}$	$0.508 \cdot C_{w,total}$	BaP concentration dissolved in water
T	°C	10 *(model input)*	water temperature
C_{ox}	mg $O_2 \cdot L^{-1}$	9.4 *(model input)*	dissolved oxygen concentration in water
lipid	—	0.05 *(model input)*	lipid content of fish (fraction of body weight)
w_w(0)	kg	0.03/0.05 *(model input)*	initial body wet weight (as in the *in vivo* study by Gerhart and Carlson, 1978[4])
w_w	kg	Equation 1	body wet weight including growth rate
K	—	for T > 10 °C $K = 3.05 \cdot 10^{-4}$ [5]	constant in equation S18
n	—	for T > 10 °C, n = 1.855 [5]	constant in equation S18
m	—	for T > 10 °C, m = -0.138 [5]	constant in equation S18
δ_b	—	0.147[6]	nonlipid organic matter of blood tissue (fraction of weight)
γ_b	—	0.839[6]	water content of blood tissue (fraction of tissue weight)
α_b	—	0.014[6]	lipid content of blood tissue (fraction of tissue weight)
α_f	—	0.942[6]	lipid content of fat tissue (fraction of tissue weight)
α_k	—	0.052[6]	lipid content of kidney tissue (fraction of tissue weight)
α_l	—	0.045[6]	lipid content of liver tissue (fraction of tissue weight)
α_g	—	0.045[6]	lipid content of guts tissue (fraction of tissue weight)
α_r	—	0.045[6]	lipid content of richly perfused tissue (fraction of tissue weight)
α_m	—	0.030[6]	lipid content of muscle tissue (fraction of tissue weight)
Q_c	$L \cdot h^{-1}$	Equation 2 [7]	cardiac output
Q_{bile}	$L \cdot h^{-1}$	$8.3 \cdot 10^{-5} \cdot w\_w$	bile flow rate
Q_l	$L \cdot h^{-1}$	$0.029 \cdot Q_c$	blood flow to the liver compartment
Q_f	$L \cdot h^{-1}$	$0.085 \cdot Q_c$	blood flow to the fat compartment
Q_m	$L \cdot h^{-1}$	$0.600 \cdot Q_c$	blood flow to the poorly perfused compartment (mainly white muscle)
Q_r	$L \cdot h^{-1}$	$0.055 \cdot Q_c$	blood flow to the richly perfused compartment
Q_g	$L \cdot h^{-1}$	$0.175 \cdot Q_c$	blood flow to the guts compartment
Q_k	$L \cdot h^{-1}$	$0.056 \cdot Q_c$	blood flow to the kidney compartment
VO_2	mg $O_2 \cdot h^{-1}$	Equation 3[5]	oxygen consumption rate for 1kg fish
Q_w	$L \cdot h^{-1}$	Equation 4	effective respiratory volume
$lipid_l$	—	Equation 5	lipid content of lean tissues (fraction of tissue weight)
$V_{digesta}$	L	$0.040 \cdot w\_w$	volume of material in the gut lumen (for fecal egestion)
V_l	L	$0.012 \cdot w\_w$	volume of the liver compartment
V_r	L	$0.015 \cdot w\_w$	volume of the richly perfused compartment
V_g	L	$0.048 \cdot w\_w$	volume of the guts compartment
V_k	L	$0.009 \cdot w\_w$	volume of the kidney compartment
V_f	L	Equation 6	volume of the fat compartment
$V_{m\_initial}$	L	$0.818 \cdot w\_w$[8]	Initial volume of poorly perfused compartment (mainly white muscle)[a]
V_m	L	Equation 7	volume of poorly perfused compartment (mainly white muscle)
P_{bw}	—	Equation 8[6]	chemical blood:water partition coefficient
P_{bl}	—	0.7[9]	chemical bile:liver partition coefficient
$P_{dg,BaP}$	—	2.62	BaP digesta:water partition coefficient calculated from[9]
P_l, P_r, P_g	—	3[8]	liver:blood (P_l) richly perfused tissue:blood and guts:blood partition coefficients of a chemical
P_k	—	2.9[8]	chemical kidney:blood partition coefficient
P_m	—	2.9[8]	chemical muscle:blood partition coefficient
P_f	—	Equation 9[9]	chemical fat:blood partition coefficient
A_f, A_m	µg	Equation 10[6]	chemical amount in fat (A_f) and poorly perfused (A_m) compartments
A_r	µg	Equation 11	chemical amount in richly perfused (A_r) compartment

D_{BaP}	—	0.2624	digestibility term for BaP (calculated for BaP from [9])
k_{gut}	$L \cdot h^{-1}$	Equation 12	gut diffusion constant
$Q_{digesta}$	$L \cdot h^{-1}$	Equation 13	fecal egestion rate
$A_{digesta}$	µg	Equation 14	chemical amount in the digesta
A_g	µg	Equation 15	chemical amount in the guts compartment
A_l	µg	Equation 16	chemical amount in the liver compartment
A_k	µg	Equation 17	chemical amount in the kidney compartment
C_{int}	µg	Equation 18	average concentration of the chemical in the whole organism
C_{art}	$\mu g \cdot L^{-1}$	Equation 19	chemical concentration in arterial blood
C_{ven}	$\mu g \cdot L^{-1}$	Equation 20	chemical concentration in venous blood
BIOTRANSFORMATION DATA			
C_{BaP}	$\mu mol \cdot L^{-1}$	from *in vitro* experiment	BaP concentration in *in vitro* system at time t (h)
C_0	$\mu mol \cdot L^{-1}$	1.6	starting concentration of the BaP
$N_{cell,\ liver}$	10^6 cells	3.186	average RTL-W1 cell number measured in *in vitro* experiment
$N_{cell,\ guts}$	10^6 cells	4.094	average RTgut-GC cell number measured in *in vitro* experiment
$N_{cell,\ richly}$	10^6 cells	8.979	average RTgill-W1 cell number measured in *in vitro* experiment
$D_{cell\text{-}liver}$	µm	16.6	average RTL-W1 cell diameter measured in *in vitro* experiment
$D_{cell\text{-}guts}$	µm	17.3	average RTgut-GC cell diameter measured in *in vitro* experiment
D_{cell_richly}	µm	15.1	average RTgill-W1 cell diameter measured in *in vitro* experiment
V_{medium}	mL	9.5	medium volume used in *in vitro* experiment
k_i	h^{-1}	Equations 21-22	biotransformation reaction rate constant which is equivalent to the regression slope for the respective model compartment
$T_{cell,liver}$	10^6 cells $\cdot$ g^{-1}	415, Equation 23	cell number in 1 g of the liver tissue
$T_{cell,guts}$	10^6 cells $\cdot$ g^{-1}	366, Equation 23	cell number in 1 g of the guts tissue
$T_{cell,richly}$	10^6 cells $\cdot$ g^{-1}	556, Equation 23	cell number in 1 g of the richly perfused tissue
$CL_{IN\ VIVO,i}$	$L \cdot h^{-1}$	Equation 24	in vivo intrinsic clearance
$f_{U,i}$	—	Equations 25	binding correction term

3. b. Equations and notes

Body wet weight (w_w) can be obtained from initial body weight (w_w(0)) and growth rate (G) calculated as in Arnot et al.:

$$G = 0.0005 \cdot w\_w^{-0.2},\ \frac{1}{d} \quad \textbf{(eq. 1)}$$

Cardiac output

$$Q_c = (0.23 \cdot T - 0.78) \cdot \left(\frac{1000 \cdot w\_w}{500}\right)^{-0.1} \cdot w\_w^{0.75},\ \frac{L}{h} \quad \textbf{(eq. 2)}$$

Oxygen consumption rate

$$VO_2 = K \cdot \left(32 + T \cdot \frac{9}{5}\right)^n \cdot \left(\frac{w\_w}{0.4536}\right)^m \cdot \frac{10000}{24},\ \frac{mgO_2}{h} \quad \textbf{(eq. 3)}$$

Effective respiratory volume

$$Q_w = \frac{VO_2}{C_{ox} - 0.2 \cdot C_{ox}} \cdot w\_w^{0.75},\ \frac{L}{h} \quad \textbf{(eq. 4)}$$

Lipid content of lean tissues

$$lipid_l = \frac{V_l \cdot \alpha_l + V_r \cdot \alpha_r + V_{m\_initial} \cdot \alpha_m + V_k \cdot \alpha_k + V_g \cdot \alpha_g}{V_l + V_r + V_{m\_initial} + V_k + V_g} \quad \textbf{(eq. 5)}$$

Volume of fat compartment

$$V_f = w\_w \cdot \frac{lipid - lipid_l}{\alpha_f - lipid_l},\ L \quad \textbf{(eq. 6)}$$

NOTE: This assumption will not work if lipid content of whole body is lower than lipid content of lean tissue (which is assumed to be independent of whole body lipid content).

Volume of poorly perfused (muscle) compartment

$$V_m = w\_w - (V_l + V_k + V_f + V_r + V_g),\ L \quad \textbf{(eq. 7)}$$

Blood:water partition coefficient of a chemical with log $K_{OW} > 3$ [10]

$$P_{bw} = (1 - \gamma_b) \cdot 10^{0.73 \cdot \log K_{OW}} + \gamma_b \quad \textbf{(eq. 8)}$$

Fat:blood partition coefficient of a chemical

$$P_f = \frac{10^{\log K_{OW}}}{P_{bw}} \quad \textbf{(eq. 9)}$$

Chemical amount in the fat and poorly perfused compartments (A_f or $A_r \rightarrow A_{f,m}$)

$$\frac{dA_{f,m}(t)}{dt} = Q_{f,m} \cdot \left(C_{art}(t) - \frac{A_{f,m}(t)}{V_{f,m} \cdot P_{f,m}} \right), \frac{\mu g}{h} \quad \textbf{(eq. 10)}$$

Chemical amount in the richly perfused compartment

$$\frac{dA_r(t)}{dt} = Q_r \cdot \left(C_{art}(t) - \frac{A_r(t)}{V_r \cdot P_r} \right) - CL_{IN\ VIVO,r} \cdot \frac{A_r}{V_r \cdot P_r}, \frac{\mu g}{h} \quad \textbf{(eq. 11)}$$

Gut diffusion rate constant (for dietary elimination route):

$$k_{gut} = \frac{1.22}{24} \cdot \left(3 \cdot 10^{-7} \cdot 10^{logK_{OW}} + 2 \right)^{-1} \cdot w\_w^{0.75}, \frac{L}{h} \quad \textbf{(eq. 12)}$$

Fecal egestion rate (for dietary elimination route):

$$Q_{digesta} = D \cdot 0.022 \cdot w\_w^{0.85} \cdot \exp(0.06 \cdot T), \frac{1}{h} \quad \textbf{(eq. 13)}$$

Chemical amount in the digesta (for dietary elimination route):

$$\frac{dA_{digesta}(t)}{dt} = 0 - Q_{digesta} \cdot \frac{A_{digesta}}{V_{digesta}} - k_g \cdot \left(\frac{A_{digesta}}{V_{digesta}} - \frac{A_g(t)}{V_g \cdot P_g} \cdot P_{dg,BaP} \right) + Q_{bile} \cdot P_{bl} \cdot P_l \cdot \frac{A_l(t)}{V_l \cdot P_l}, \frac{\mu g}{h} \quad \textbf{(eq. 14)}$$

NOTE: "0" accounts for no chemical dietary uptake

Chemical amount in the guts compartment

$$\frac{dA_g(t)}{dt} = Q_g \cdot \left(C_{art}(t) - \frac{A_g(t)}{V_g \cdot P_g} \right) + k_g \cdot \left(\frac{A_{digesta}}{V_{digesta}} - \frac{A_g(t)}{V_g \cdot P_g} \cdot P_{dg,BaP} \right) - CL_{IN\ VIVO,r} \cdot \frac{A_g}{V_g \cdot P_g}, \frac{\mu g}{h} \quad \textbf{(eq. 15)}$$

Chemical amount in the liver compartment

$$\frac{dA_l(t)}{dt} = Q_g \cdot \frac{A_g(t)}{V_g \cdot P_g} + Q_l \cdot C_{art}(t) - (Q_g + Q_l + CL_{INVIVO,l}) \cdot \frac{A_l(t)}{V_l \cdot P_l}, \frac{\mu g}{h} \quad \textbf{(eq. 16)}$$

Chemical amount in the kidney compartment

$$\frac{dA_k(t)}{dt} = 0.6 \cdot Q_m(t) \cdot \frac{A_m(t)}{V_m \cdot P_m} + Q_k \cdot C_{art}(t) - (0.6 \cdot Q_m + Q_k + CL_{INVIVO,k}) \cdot \frac{A_k(t)}{V_k \cdot P_k}, \frac{\mu g}{h} \quad \textbf{(eq. 17)}$$

NOTE: by default, the initial chemical amount in each tissue should be equal to zero (i.e. Ai(0) = 0).

Chemical internal concentration in the whole body of rainbow trout

$$Cint(t) = \frac{A_f(t) + A_m(t) + A_r(t) + A_l(t) + A_k(t) + A_g(t)}{1000 \cdot w\_w}, \frac{\mu g}{g} \quad \textbf{(eq. 18)}$$

Chemical concentration in arterial blood

$$Cart(t) = \min(Q_w, Q_c \cdot P_{bw}) \cdot \frac{C_w - \frac{C_{ven}(t)}{P_{bw}}}{Q_c} + C_{ven}(t), \frac{\mu g}{L} \quad \textbf{(eq. 19)}$$

Chemical concentration in venous blood **(eq. 20)**

$$Cven(t) = \frac{Q_f \cdot \frac{A_f(t)}{V_f \cdot P_f} + Q_r \cdot \frac{A_r(t)}{V_r \cdot P_r} + 0.4 \cdot Q_m \cdot \frac{A_m(t)}{V_m \cdot P_m} + (0.6 \cdot Q_m + Q_k) \cdot \frac{A_k(t)}{V_k \cdot P_k} + (Q_g + Q_l) \cdot \frac{A_l(t)}{V_l \cdot P_l}}{Q_c}, \frac{\mu g}{L}$$

<u>Biotransformation data</u>

The biotransformation rate constant could be described by the first order elimination rate which was derived from measured concentrations of the parent chemical, using ln-transformation as follows:

$$\frac{dC_{BaP}}{dt} = -k_i \cdot C_{BaP}(t) \rightarrow C_{BaP}(t) = C_o + e^{-k_i t} \rightarrow \ln(C_{BaP}(t)) = \ln(C_o + e^{-k_i t}) \textbf{(eq.21)}$$

thus:

$$\ln C_{BaP} = \ln C_0 - k_i \cdot t \quad [\mu M], \quad \textbf{(eq.22)}$$

NOTE: These calculations are in agreement with the reaction rate constants determined based on $\log_{10}$-transformation of the parent compound, presented by Han et al.,[11] who presented the slope as (-k)/2.3 and 2.3 ≈ ln(10), where 10 is a base of the used logarithm.

The cell number predicted to amount to 1 g of respective tissue can be taken from the literature or determined based on the cells' diameter *D* (µm) as follows:

$$T_{cell} = \frac{1}{\frac{4}{3}\cdot\pi\cdot\left(\frac{D_{cell}}{2}\cdot\frac{1}{100}\right)^3}, \left[\frac{10^6\,\text{cells}}{\text{g}}\right] \quad \textbf{(eq. 23)}$$

NOTE: T_{cell} for hepatocytes was measured between 420 and 540 × 10^6 cells per 1 g of liver and can be set to 500 × 10^6 cells/g as done for hepatocytes[12], while for RTL-W1 cell line, RTgutGC and RTgill-W1, 415, 366 and 556 × 10^6 cells per respective cell type were measured. The similarities of values given above between hepatocytes and cell lines support that assumption that they are of comparable size. The values for the RTgill-W1 cell line were furthermore applied to represent all richly perfused tissues (aside from liver and intestine) in an attempt to account for biotransformation in the entire organism.

In vivo intrinsic clearance:

$$CL_{IN\ VIVO} = \frac{Q_{tissue}\cdot f_U\cdot\frac{k_i}{\frac{N_{cell}}{V_{medium}}}\cdot T_{cell}\cdot W_{tissue}}{Q_{tissue}+f_U\cdot\frac{k_i}{\frac{N_{cell}}{V_{medium}}}\cdot T_{cell}\cdot W_{tissue}}, \quad [\text{L/h}] \quad \textbf{(eq. 24)}$$

Binding correction term:

$$f_U = \frac{\frac{\gamma_B}{P_{bw}}}{\frac{C_{cell}}{2\cdot10^6}\cdot10^{0.676\cdot\log K_{OW}-2.215}+1}, [-] \quad \textbf{(eq. 25)}$$

(4) Results

4. a. Cell viability of rainbow trout gill, gut and liver cells upon exposure to BaP for 1, 2, 3 and 11 days

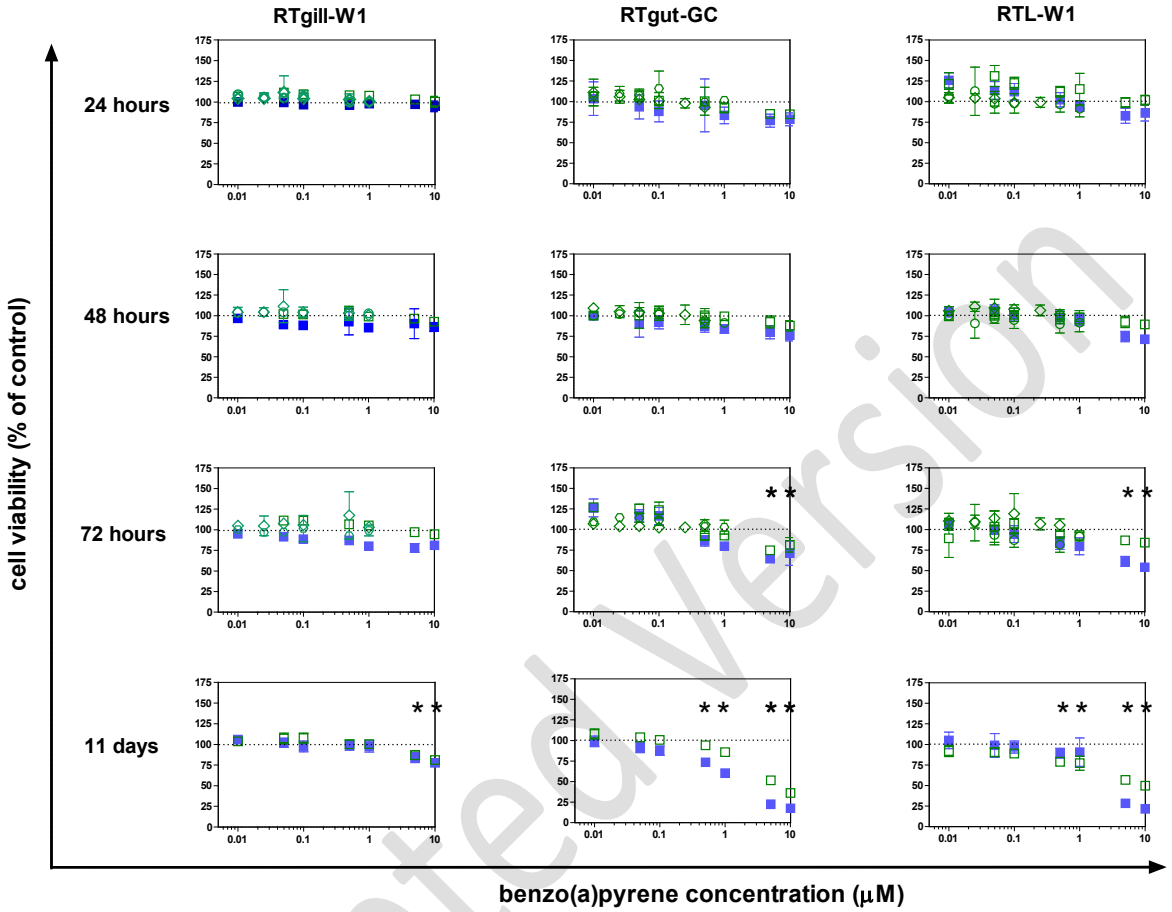


Figure S1. Cell viability of rainbow trout gill, gut and liver cells upon exposure to BaP for 1, 2, 3 and 11 days with test concentrations ranging from 0.01 to 10 µM. Cell viability was assessed as metabolic activity (alamarBlue; filled blue squares) and plasma membrane integrity (CFDA-AM; empty green squares), expressed as % of control (indicated by dashed line). Square symbols for both metabolic activity and plasma membrane integrity stem from the same dedicated cell viability experiment. The other symbols (empty hexagon and rhombus) originate from two independent experiments focusing on EROD activity (see Figure 1 in the main manuscript) in which CFDA-AM measurements were taken simultaneously. Each symbol represents the average and standard deviation of five to six culture wells from one independent experiment. Cell viability significantly declined for both viability measures in RTgutGC and RTL-W1 cell lines at 5 and 10 µM BaP > 48 hours of exposure and > 0.1 µM BaP at 11 days whereas significant differences were found only on day 11 and only for the two highest BaP concentrations in RTgill-W1 (ANOVA, followed by Dunnett's test, $p < 0.05$, marked by *).

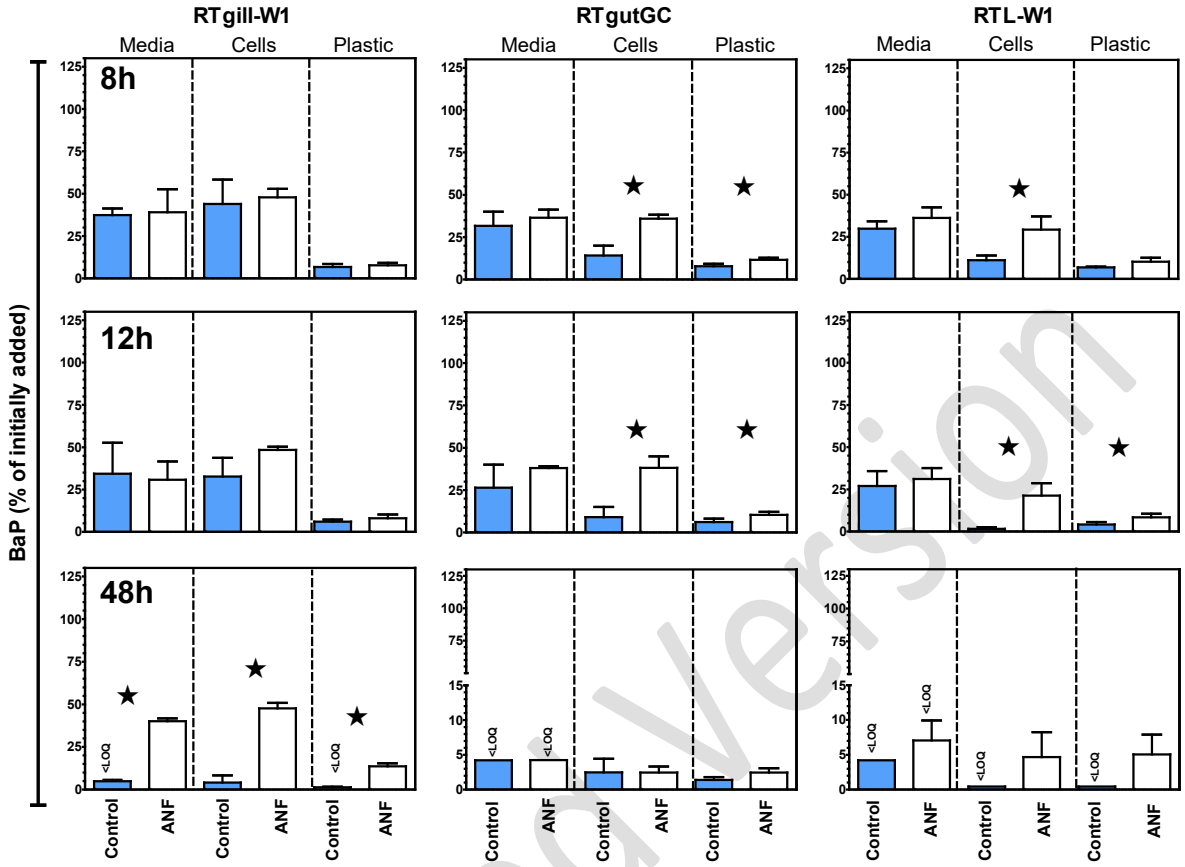
4. b. Data for time-dependent distribution of BaP in culture flasks

Table S-2. Time-dependent distribution of BaP in culture flasks containing either RTL-W1, RTgutGC or RTgill-W1 cells. At each indicated exposure time, the cells, the culture medium and the plastic of the flasks were extracted and analyzed for non-biotransformed BaP to obtain a parent compound mass balance. The difference between total amount recovered and initial amount added was assigned to the % biotransformed. BaP recovery in cell-free control experiments ranged from 94-112% (see Materials and Methods in main manuscript). Values given are averages of flasks of three independent experiments and their standard deviation. The limit of quantification (LOQ) amounted to 0.02 µmol/L for the plastic and cell fraction and to 0.2 µmol/L for the medium.

Exposure time	Initial measured amount of B[a]P measured by Radio HPLC [µmol/L]			Method	Radio-HPLC RTgill-W1		Radio-HPLC RTgut-GC		Radio-HPLC RTL-W1	
[h]	RTgill-W1	RTgutGC	RTL-W1	Fraction	B[a]P [µmol/L] (1)	B[a]P [%] (2)	B[a]P [µmol/L (1)	B[a]P [%] (2)	B[a]P [µmol/L] (1)	B[a]P [%] (2)
6	1.60 ± 0.21	1.57 ± 0.09	1.77 ± 0.03	Media	0.92 ± 0.08	57.45 ± 5.13	1.27 ± 0.53	81.08 ± 34.01	0.86 ± 0.07	48.46 ± 4.21
				Cells	0.51 ± 0.07	32.04 ± 4.43	0.28 ± 0.06	18.01 ± 3.93	0.36 ± 0.03	20.28 ± 1.60
				Plastic	0.11 ± 0.02	6.83 ± 1.24	0.17 ± 0.02	10.86 ± 1.23	0.11 ± 0.01	6.48 ± 0.69
				Biotransformation (2)	0.06 ± 0.21	3.69 ± 13.20	-0.16 ± 0.50	-9.96 ± 32.06	0.44 ± 0.04	24.78 ± 2.13
8	1.58 ± 0.16	1.36 ± 0.08	1.33 ± 0.14	Media	0.59 ± 0.06	37.41 ± 3.90	0.43 ± 0.11	31.70 ± 8.37	0.40 ± 0.06	29.82 ± 4.40
				Cells	0.69 ± 0.23	43.99 ± 14.33	0.19 ± 0.08	14.21 ± 5.81	0.15 ± 0.04	11.09 ± 2.84
				Plastic	0.11 ± 0.03	6.68 ± 1.85	0.11 ± 0.02	7.87 ± 1.38	0.09 ± 0.01	6.88 ± 0.41
				Biotransformation (2)	0.19 ± 0.06	11.91 ± 3.86	0.63 ± 0.20	46.22 ± 14.53	0.69 ± 0.16	52.21 ± 11.89
12	1.55 ± 0.28	1.60 ± 0.27	1.40 ± 0.24	Media	0.53 ± 0.28	34.37 ± 18.28	0.42 ± 0.22	26.43 ± 13.67	0.38 ± 0.12	26.97 ± 8.89
				Cells	0.51 ± 0.17	32.64 ± 11.09	0.15 ± 0.10	9.09 ± 5.98	0.02 ± 0.01	1.65 ± 0.99
				Plastic	0.09 ± 0.02	5.98 ± 1.34	0.10 ± 0.03	6.18 ± 1.98	0.06 ± 0.02	4.42 ± 1.42
				Biotransformation (2)	0.42 ± 0.28	27.01 ± 18.09	0.93 ± 0.22	58.31 ± 13.80	0.94 ± 0.10	67.19 ± 7.08
24	1.62 ± 0.32	1.51 ± 0.20	1.59 ± 0.39	Media	0.33 ± 0.17	20.11 ± 10.38	<LOQ	<LOQ	<LOQ	<LOQ
				Cells	0.22 ± 0.17	13.62 ± 10.23	0.07 ± 0.03	4.56 ± 1.96	0.03 ± 0.03	2.05 ± 1.64
				Plastic	0.06 ± 0.05	3.78 ± 2.88	0.05 ± 0.02	3.13 ± 1.57	0.03 ± 0.01	1.66 ± 0.64
				Biotransformation (2)	1.01 ± 0.63	62.49 ± 39.03	1.40 ± 0.21	92.31 ± 13.79	1.53 ± 0.36	96.29 ± 22.56
48	1.48 ± 0.23	1.51 ± 0.20	1.51 ± 0.38	Media	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
				Cells	0.06 ± 0.06	4.13 ± 4.19	0.04 ± 0.03	2.46 ± 1.96	<LOQ	<LOQ
				Plastic	<LOQ	<LOQ	0.02 ± 0.01	1.38 ± 0.43	<LOQ	<LOQ
				Biotransformation (2)	1.42 ± 0.29	95.87 ± 19.87	1.45 ± 0.17	96.16 ± 11.58	1.51 ± 0.38	100.00 ± 25.38
72	1.57 ± 0.24	1.50 ± 0.18	1.61 ± 0.14	Media	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
				Cells	<LOQ	<LOQ	0.02 ± 0.03	1.49 ± 1.72	<LOQ	<LOQ
				Plastic	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
				Biotransformation (2)	1.57 ± 0.24	100.00 ± 15.13	1.48 ± 0.16	98.51 ± 10.52	1.61 ± 0.14	100.00 ± 8.95

(1) Measured value of B[a]P amount per each fraction in nmol (average n=3 ± SD); the values of the radio-HPLC were converted from DPM into µmol/L.
(2) Amount of B[a]P of all the fractions (in %) and the biotransformed B[a]P amount (in µM/L and %) per exposure test flask were evaluated by the initial amount of B[a]P (average n=3 ± SD).

4. c. Time-dependent distribution of BaP in culture flasks in the presence or absence of the inhibitor ANF



cell lines in the presence or absence of the inhibitor ANF. Empty bars represent the results in the presence of ANF; filled bars represent the results in the absence of ANF (Control). At each indicated exposure time, the cells, the culture medium and the plastic of the flasks were extracted and analyzed for BaP to obtain a parent compound mass balance. Values given are averages of flasks of three independent experiments. Stars indicate significant difference between control and ANF treated flasks based on unpaired, two-sided t-test. LOQ: Limit of Quantification.

5. References

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