Toxicokinetic Modeling of Per- and Polyfluoroalkyl Substance Concentrations within Developing Zebrafish (*Danio rerio*) Populations

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Supporting Information

Number of pages: 51
Number of Figures: 5
Number of Tables: 3
# Table of Contents

Volume model derivation.............................................................................................................. S3
Surface area model...................................................................................................................... S6
Hatching fraction model............................................................................................................... S7
Fitting procedure for toxicokinetic models.................................................................................. S8
Vogs et al\(^1\) model equations (2 compartment model)............................................................. S9
Commentary on experimental differences of literature studies used for data......................... S10
Table S1: Model parameters used in this work........................................................................... S11
Table S2: Model parameters used in Vogs et al\(^1\)................................................................... S13
Table S3: Experimental differences of literature studies used for data....................................... S15
Figure S1: Auxiliary model fits................................................................................................... S17
Figure S2: Kinetic, transporter, and Vogs et al\(^1\) model fits....................................................... S18
Figure S3: Biphasic uptake discussion......................................................................................... S21
Figure S4: Chorion intact vs. dechorionated comparison......................................................... S22
Figure S5: Compartment-by-compartment mass distribution investigation.............................. S23
Modeling code: Kinetic form for PFOA....................................................................................... S24
Modeling code: Kinetic form for PFOS....................................................................................... S29
Modeling code: Functions for the kinetic form.......................................................................... S34
Modeling code: Transporter form for PFOA................................................................................ S36
Modeling code: Transporter form for PFOS................................................................................ S42
Modeling code: Functions for the transporter form.................................................................... S48
References.................................................................................................................................... S50
Volume model derivation

We begin by deriving an equation for the yolk (subscript \(Y\)). Under the assumption of a constant mass transfer rate to the embryo from the yolk, we write the yolk mass balance as:

\[
\frac{d(\rho_Y V_Y)}{dt} = -R_Y V_Y, \quad S1a
\]

wherein \(\rho\) is a density, \(V\) is a volume, \(R_Y\) is the constant mass transfer rate to the embryo, and \(t\) is time. The initial time is at 0 hours post fertilization (hpf) or the beginning of the zygote period. Since we’re assuming the yolk density is constant, we can divide by density, resulting in:

\[
\frac{dV_Y}{dt} = -K_Y V_Y, \quad S1b
\]

wherein \(K_Y\) is the constant mass transfer rate to the embryo with different units than \(R_Y\). Equation S1b then has the trivial analytical solution of:

\[
V_Y = V_Y^0 \exp(-K_Y t), \quad S1c
\]

wherein superscript naught (\(^0\)) refers to the initial condition.

For the embryo volume (subscript \(E\)), we write the mass balance as:

\[
\frac{d(\rho_E V_E)}{dt} = -\frac{d(\rho_Y V_Y)}{dt}. \quad S2a
\]

Unlike the yolk, we do not assume that the embryo density is constant, as many different cell/tissue types and anatomical features, such as the swim bladder, are forming, resulting in the mass balance:

\[
\rho_E \frac{dV_E}{dt} + V_E \frac{d\rho_E}{dt} = -\rho_Y \frac{dV_Y}{dt}. \quad S2b
\]

From this, we posit that the ratio of yolk to embryo densities (\(\bar{\rho}\)) can be described by a well-behaved function of time. Writing Equation S2b in terms of \(\bar{\rho}\) results in:

\[
\frac{dV_E}{dt} = \bar{\rho} \left( -\frac{dV_Y}{dt} - V_E \frac{d(1/\bar{\rho})}{dt} \right). \quad S2c
\]

A hint as to the form of \(\bar{\rho}\) can be obtained by plotting the ratio of the embryo volume differential to the negative of the yolk volume differential (not shown), as found through the application of
finite differencing to the dataset. As such, we proposed an empirically motivated exponential function to describe $\bar{\rho}$:

$$\bar{\rho} = \bar{\rho}^o \exp(K_p t), \quad S2d$$

wherein $\bar{\rho}^o$ and $K_p$ are the pre-exponential and rate constant of the exponential function, respectively. Equations S2c and S2d can then be combined, along with Equations S1b and S1c, to write the final form of the embryo volume differential equation:

$$\frac{dV_E}{dt} = \bar{\rho}^o K_Y V_Y^p \exp\left((K_p - K_Y) t\right) + K_p V_E. \quad S2e$$

Equation S2e then has the nontrivial analytical solution of:

$$V_E(t) = \exp(K_p t) \left(V_Y^p + \bar{\rho}^o V_Y^p (1 - \exp(-K_Y t))\right). \quad S2f$$

With expressions for the yolk and embryo volumes identified, we must address the final compartment of the system, that of the perivitelline space. The perivitelline space (labeled as the chorion (subscript $C$) for brevity) is spatially bound by both the chorion and the embryo/yolk complex and is present only before the chorion is shed during the hatching period. To describe the volume of this space for the pre-hatching phase, we need to describe the size of the chorion through time. In the literature, there is a wide range of diameters reported for the spherically-shaped membrane, with OECD Test Guideline 236 reporting a diameter of approximately 0.8-1.5 mm. We confirmed this range by examining the literature for images from the pre-hatch period, in which the chorion remained intact over the experimental duration. From image analysis of these studies, we obtained an approximate range between 0.9-1.3 mm, consistent with OECD Test Guideline 236, and we found that the diameter of a given chorion is relatively constant through time. These studies did not include images at 0 hpf, so we adopted the diameter found in Kimmel et al. for 0 hpf, that of 1 mm, to set the constant pre-hatch spherical volume of the system. With the volume of the system set, combined with the known equations of the yolk and embryo volumes, we can write the volume of the perivitelline space either in differential form:

$$\frac{dV_C}{dt} = -\frac{dV_Y}{dt} - \frac{dV_E}{dt}, \quad S3a$$

or algebraic form:

$$V_C = V_{sys} - V_Y - V_E, \quad S3b$$

wherein subscript $sys$ refers to the total system.
The parameters of Equations S1-S2 (including the initial yolk volume) were fit to the Simeon et al. dataset using a conventional least squares approach using MATLAB’s (R2020b; The MathWorks, Natick, MA) “fminsearch” algorithm and can be found in Table S1. The fits can be found in Figure S1. For the PFAS transport analysis we combined the embryo and yolk compartments into one compartment, termed the embryo, as the PFAS datasets used in this study are only resolved enough to warrant two compartment resolution (chorion and embryo).
**Surface area model**

The surface area of the chorion is a constant from our constant chorion diameter assumption. However, the embryo is more complicated, and the prevalence of embryo surface area data is even scarcer than that of volume. The best dataset we found comes from Guo et al for hatched embryos between 72-120 hpf. They also provide volume data for the same time period that is in reasonable agreement with Simeon et al. We leveraged this fact to develop our surface area expression as a function of volume. Specifically, we found that a linear correlation between the surface area and volume of Guo et al provided a good fit, such that:

\[ A_E = \frac{V_E}{\lambda}, \]

wherein \( A_E \) is the embryo surface area and \( \lambda \) is the fitted ratio of embryo volume to surface area. This equation not only provides a good fit for the 72-120 hpf window but it also provides a reasonable approximation for times < 72 hpf. At earlier times, the embryo can be approximated with spherical geometry, as done by Brox et al. Our linear correlation captures a spherical volume to surface area conversion at earlier times (smaller volumes) but then matches the data of Guo et al for later times where a spherical correlation would underpredict surface area.

The parameter of Equation S4 was fit to the Guo et al dataset using MATLAB’s “lsqcurvefit” algorithm and can be found in Table S1. The fit can be found in Figure S1.
Hatching fraction model

The dataset for our hatching fraction model is found in Hagenaars et al\textsuperscript{10}. They recorded hatching fraction as a function of time for several PFOA concentrations; however, as with our volume and subsequent surface area model, we did not consider PFAS concentration dependence on the hatching fraction. From a survey of the control zebrafish population reported in Hagenaars et al\textsuperscript{10}, a sigmoid curve reasonably captures hatching phenomenology over time, resulting in:

\[ h = \frac{t^n}{\tau^n + t^n}, \]

wherein \( h \) is the hatched fraction, \( \tau \) is the time at which half of the population has hatched, and \( n \) is an exponent that adjusts the maximum hatching rate across the population. Here, we have assumed perfect hatching where the minimum and maximum hatched fraction are 0 and 1, respectively. Parameters were fit to the Hagenaars et al\textsuperscript{10} dataset using MATLAB’s “lsqcurvefit” algorithm and can be found in Table S1. The fit can be found in Figure S1.
Fitting procedure for toxicokinetic models

Parameters for our toxicokinetic models were fit using a modified least squares approach. Due to the digitization process of the Vogs et al. dataset, not all data points were able to be captured, resulting in an unequal number of data points between the three exposure concentrations. As such, we normalized the residual sum of squares for each concentration against its respective number of data points to weight each exposure concentration more evenly in the fitting procedure. We further weighted the contribution of each exposure concentration to the residual sum of squares via log-normalization.

Overall, several apparent local minima were found for this fitting problem. A best-fit approach was employed where MATLAB’s “multistart” function was called to run the gradient descent solver of “fmincon” across a custom grid of hundreds of initial parameter guesses, with “ode15s” called to numerically solve the coupled differential equations. Parameters were constrained on the interval \([0, \infty)\), and the best-fit parameters can be found in Table S1.
Vogs et al. model equations (2 compartment model)

Chorion:
\[
\frac{dC_C}{dt} = k_{WC} \cdot C_W - k_{CW} \cdot C_C + k_{EC} \cdot C_E - k_{CE} \cdot C_C, \quad t \leq 48 \text{ hpf}
\]

Embryo:
\[
\frac{dC_E}{dt} = k_{CE} \cdot C_C - k_{EC} \cdot C_E, \quad t \leq 48 \text{ hpf}
\]
\[
\frac{dC_E}{dt} = k_{WE} \cdot C_W - k_{EW} \cdot C_E, \quad t > 48 \text{ hpf}
\]

wherein \( k \) is a kinetic rate constant between two locations [1/hr], and the rest of the variables and subscripts are defined in the main text.
Commentary on experimental differences between the literature studies used for data

In Table S3, we compare some key experimental variables across the studies when considering PFAS exposure, but it is not entirely clear why the PFOA data is so variable in comparison to PFOS. The biggest unknown from some studies is whether the study is actually keeping a constant exposure concentration, as although some studies replace exposure solutions daily, they do not provide exposure concentration measurements throughout the entire duration. It is an unknown how much of an impact this has on measurements, but measured concentrations are usually lower than nominal concentrations, and the biggest discrepancy with the PFOA data of Figure 1 is lower measured concentrations than expected.

From Figure 5, we expect some bioconcentration of PFOA around 5 and 6 dpf. As mentioned above, most literature values of Figure 1 have a lower than expected measured concentration, which also indicates lower than expected bioconcentration. For example, the Wang et al11’ and Gaballah et al12’ datasets have a much lower bioconcentration than expected given the Vogs et al’ calibration dataset. Specifically, the lowest exposure concentration from Wang et al11’ is 92.8 µM with an 84.9 µM measured concentration and a 537.1 µM theoretical concentration at 5 dpf. Similarly, the lowest exposure concentration for Gaballah et al12’ is 23.9 µM with a 26.8 µM measured concentration and a 219.2 µM theoretical concentration at 6 dpf. Both of these studies show minimal bioconcentration, which is unexpected considering other fish studies have reported on non-negligible bioconcentration (see Table 2 of Vogs et al’). This low bioconcentration is in contrast to the Han et al13’ data point for which our model prediction is within a factor of 2: exposure concentration of 3.94 µM with an 88.9 µM measured concentration and a 45.4 µM theoretical concentration at 5 dpf. Overall, there appears to be a large discrepancy in the results some studies are obtaining when considering the bioconcentration of PFOA, leading to our model not being able to predict the data of the validation studies well. Moving past the compiled experimental variables in Table S3, we do hypothesize that the underprediction of the Menger et al14’ data (both PFOA and PFOS) is most likely attributable to the fact that the Menger et al14’ study obtained the data from a mixture experiment (i.e., the zebrafish was exposed to a PFAS mixture and not a single PFAS exposure, confounding the data due to potential unknown system interactions with the mixture and interactions amongst the components of the mixture).
**Table S1.** A comprehensive list of parameters from all models used in this study. All parameters, except for $V_{sys}$ and $V_E^0$, were found through data fitting.

| Parameter       | Description                                              | Value          |
|-----------------|----------------------------------------------------------|----------------|
| $V_{sys}$       | Total system volume (literature estimate) [L]             | 5.24e-7        |
| $V_E^0$         | Initial embryo volume (literature estimate, small value set to zero) [L] | 0              |
| $V_Y^0$         | Initial yolk volume [L]                                  | 1.45e-7        |
| $\bar{\rho}$   | Pre-exponential of density ratio function [unitless]      | 1.70           |
| $K_p$           | Rate constant of density ratio function [1/hr]           | 6.32e-3        |
| $K_Y$           | Mass transfer rate constant from yolk to embryo [1/hr]   | 7.73e-3        |
| $\lambda$       | Ratio of embryo volume to area [mm]                      | 0.0804         |
| $n$             | Exponent that sets maximum hatching rate                  | 19.25          |
| $\tau$          | Time at which half the population has hatched [hr]       | 62.63          |
| $k_{WC}$        | Max kinetic rate between water and chorion (PFOA) [µmol/hr/mm²] | 7.74e-7       |
| $k_{WC}$        | Max kinetic rate between water and chorion (PFOS) [µmol/hr/mm²] | 3.63e-7       |
| $k_{XE}$        | Max kinetic rate between water/chorion and embryo (PFOA) [µmol/hr/mm²] | 1.21e-6       |
| $k_{XE}$        | Max kinetic rate between water/chorion and embryo (PFOS) [µmol/hr/mm²] | 6.45e-7       |
| $K_C$           | Chorion Michaelis constant (PFOA) [µM]                  | 75.05          |
| $K_C$           | Chorion Michaelis constant (PFOS) [µM]                  | 1.30           |
| $K_E$           | Embryo Michaelis constant (PFOA) [µM]                   | 58.51          |
| $K_E$           | Embryo Michaelis constant (PFOS) [µM]                   | 1.17           |
Table S1. Continued.

| Parameter | Description | Value |
|-----------|-------------|-------|
| $k_{WC}$ | Diffusion constant between water and chorion (PFOA) [µmol*µM/hr/mm²] | 1.44e-4 |
| $k_{WC}$ | Diffusion constant between water and chorion (PFOS) [µmol*µM/hr/mm²] | 7.97e-6 |
| $k_{XE}$ | Diffusion constant between water/chorion and embryo (PFOA) [µmol*µM/hr/mm²] | 6.84e-5 |
| $k_{XE}$ | Diffusion constant between water/chorion and embryo (PFOS) [µmol*µM/hr/mm²] | 5.60e-7 |
| $K_{C}^{ads}$ | Adsorption/binding constant of chorion (PFOA) [L] | 3.44e-12 |
| $K_{C}^{ads}$ | Adsorption/binding constant of chorion (PFOS) [L] | 2.47e-9 |
| $K_{E}^{ads}$ | Adsorption/binding constant of embryo (PFOA) [L] | 4.00e-6 |
| $K_{E}^{ads}$ | Adsorption/binding constant of embryo (PFOS) [L] | 1.08e-1 |
| $K_{C}^{SC}$ | Solute-carrier equilibrium constant of chorion (PFOA) [µM] | 144.19 |
| $K_{C}^{SC}$ | Solute-carrier equilibrium constant of chorion (PFOS) [µM] | 3.28 |
| $K_{E}^{SC}$ | Solute-carrier equilibrium constant of embryo (PFOA) [µM] | 20.69 |
| $K_{E}^{SC}$ | Solute-carrier equilibrium constant of embryo (PFOS) [µM] | 1.05 |
Table S2. The list of parameters used by the Vogs et al. model.

| Parameter | Description                                               | Value   |
|-----------|-----------------------------------------------------------|---------|
| $k_{WC}$  | Kinetic rate between water and chorion (PFOA, 340 µM) [1/hr] | 0.02    |
| $k_{WC}$  | Kinetic rate between water and chorion (PFOA, 41 µM) [1/hr] | 0.10    |
| $k_{WC}$  | Kinetic rate between water and chorion (PFOA, 21 µM) [1/hr] | 0.11    |
| $k_{WC}$  | Kinetic rate between water and chorion (PFOS, 0.76 µM) [1/hr] | 1.86    |
| $k_{WC}$  | Kinetic rate between water and chorion (PFOS, 0.08 µM) [1/hr] | 3.02    |
| $k_{WC}$  | Kinetic rate between water and chorion (PFOS, 0.04 µM) [1/hr] | 1.99    |
| $k_{CW}$  | Kinetic rate between chorion and water (PFOA, 340 µM) [1/hr] | 8.47e-9 |
| $k_{CW}$  | Kinetic rate between chorion and water (PFOA, 41 µM) [1/hr] | 5.94e-9 |
| $k_{CW}$  | Kinetic rate between chorion and water (PFOA, 21 µM) [1/hr] | 3.73e-14|
| $k_{CW}$  | Kinetic rate between chorion and water (PFOS, 0.76 µM) [1/hr] | 2.86e-8 |
| $k_{CW}$  | Kinetic rate between chorion and water (PFOS, 0.08 µM) [1/hr] | 1.89e-7 |
| $k_{CW}$  | Kinetic rate between chorion and water (PFOS, 0.04 µM) [1/hr] | 3.61e-8 |
| $k_{CE}$  | Kinetic rate between chorion and embryo (PFOA, 340 µM) [1/hr] | 0.12    |
| $k_{CE}$  | Kinetic rate between chorion and embryo (PFOA, 41 µM) [1/hr] | 0.05    |
| $k_{CE}$  | Kinetic rate between chorion and embryo (PFOA, 21 µM) [1/hr] | 0.04    |
| $k_{CE}$  | Kinetic rate between chorion and embryo (PFOS, 0.76 µM) [1/hr] | 0.07    |
| $k_{CE}$  | Kinetic rate between chorion and embryo (PFOS, 0.08 µM) [1/hr] | 0.06    |
| $k_{CE}$  | Kinetic rate between chorion and embryo (PFOS, 0.04 µM) [1/hr] | 0.81    |
| $k_{EC}$  | Kinetic rate between embryo and chorion (PFOA, 340 µM) [1/hr] | 7.51e-15|
| $k_{EC}$  | Kinetic rate between embryo and chorion (PFOA, 41 µM) [1/hr] | 6.82e-15|
| $k_{EC}$  | Kinetic rate between embryo and chorion (PFOA, 21 µM) [1/hr] | 2.67e-15|
| $k_{EC}$  | Kinetic rate between embryo and chorion (PFOS, 0.76 µM) [1/hr] | 3.82e-8 |
| $k_{EC}$  | Kinetic rate between embryo and chorion (PFOS, 0.08 µM) [1/hr] | 3.95e-13|
| $k_{EC}$  | Kinetic rate between embryo and chorion (PFOS, 0.04 µM) [1/hr] | 3.42e-13|
Table S2. Continued.

| Parameter \( k_{WE} \) | Description | Value |
|-------------------------|-------------|-------|
| \( k_{WE} \) Kinetic rate between water and embryo (PFOA, 340 µM) [1/hr] | 0.02 |
| \( k_{WE} \) Kinetic rate between water and embryo (PFOA, 41 µM) [1/hr] | 0.06 |
| \( k_{WE} \) Kinetic rate between water and embryo (PFOA, 21 µM) [1/hr] | 0.18 |
| \( k_{WE} \) Kinetic rate between water and embryo (PFOS, 0.76 µM) [1/hr] | 2.50 |
| \( k_{WE} \) Kinetic rate between water and embryo (PFOS, 0.08 µM) [1/hr] | 7.02 |
| \( k_{WE} \) Kinetic rate between water and embryo (PFOS, 0.04 µM) [1/hr] | 3.60 |
| \( k_{EW} \) Kinetic rate between embryo and water (PFOA, 340 µM) [1/hr] | 1.09e-2 |
| \( k_{EW} \) Kinetic rate between embryo and water (PFOA, 41 µM) [1/hr] | 3.23e-3 |
| \( k_{EW} \) Kinetic rate between embryo and water (PFOA, 21 µM) [1/hr] | 1.46e-2 |
| \( k_{EW} \) Kinetic rate between embryo and water (PFOS, 0.76 µM) [1/hr] | 5.86e-3 |
| \( k_{EW} \) Kinetic rate between embryo and water (PFOS, 0.08 µM) [1/hr] | 2.68e-2 |
| \( k_{EW} \) Kinetic rate between embryo and water (PFOS, 0.04 µM) [1/hr] | 6.43e-3 |
Table S3. Compiled experimental variables across the studies used to extract data. For the ratio of embryo volume to exposure volume, the maximum chorion/embryo system volume of 0.524 mm$^3$ was used for each individual in an exposure solution.

| Ratio of System Volume to Exposure Volume |  |
|------------------------------------------|--|
| Vogs et al$^1$                           | 5.24e-4 |
| Menger et al$^{14}$                      | 2.10e-3 |
| Wang et al$^{11}$                        | 3.14e-3 |
| Gaballah et al$^{12}$                    | 2.10e-3 |
| Spulber et al$^{15}$                     | 6.99e-4 |
| Han et al$^{13}$                         | 3.49e-3 |
| Tu et al$^{16}$                          | Unknown |
| Huang et al$^{17}$                       | 2.62e-3 |

| Constant Exposure Concentration?         |  |
|------------------------------------------|--|
| Vogs et al$^1$                           | Yes, measurements shown |
| Menger et al$^{14}$                      | Some loss over time, measurements shown |
| Wang et al$^{11}$                        | Solutions replaced every day |
| Gaballah et al$^{12}$                    | Solutions replaced every day |
| Spulber et al$^{15}$                     | Unknown |
| Han et al$^{13}$                         | Solutions replaced every day |
| Tu et al$^{16}$                          | Yes, some measurements shown, solutions replaced every day |
| Huang et al$^{17}$                       | Yes, measurements shown |

| Max Exposure Concentration in Relation to Effect Concentration |  |
|----------------------------------------------------------------|--|
| Vogs et al$^1$                                               | Less than EC$_{20}$ |
| Menger et al$^{14}$                                         | No difference from the control |
| Wang et al$^{11}$                                           | Set at the EC$_{50}$ |
| Gaballah et al$^{12}$                                       | Much less than EC$_{50}$ |
| Spulber et al$^{15}$                                        | Resulted in a maximum of 5% mortality or embryonic effects |
| Han et al$^{13}$                                            | Not conducted, smaller max concentration than some other studies |
| Tu et al$^{16}$                                             | Less than 10% of LC$_{50}$ |
| Huang et al$^{17}$                                          | Exceeds the LC$_{50}$ |
### Table S3. Continued.

| Study | Incubation Temperature | Incubation Light |
|-------|-------------------------|------------------|
| Vogs et al | 28 ± 1 °C | Dark |
| Menger et al | Unknown | |
| Wang et al | 28 °C | 14h:10h light cycle |
| Gaballah et al | 26 °C | 14h:10h light cycle |
| Spulber et al | 28 °C | 14h:10h light cycle |
| Han et al | 28.5 °C | 14h:10h light cycle |
| Tu et al | 28.5 °C | 12h:12h light cycle |
| Huang et al | 28 ± 0.5 °C | “Light controlled” |

| Study | Exposure Container | Exposure Solution | Is it Buffered? (Y/N) |
|-------|--------------------|-------------------|----------------------|
| Vogs et al | Glass Petri dish | E3 medium | Y |
| Menger et al | Plastic Petri dish | Carbon-filtered tap water | N |
| Wang et al | 6-well | Unknown, Unknown |
| Gaballah et al | 96-well with nylon mesh insert | HBSS | Y |
| Spulber et al | 48-well | E3 water | Unknown |
| Han et al | 24 well | Unknown, Unknown |
| Tu et al | Glass beaker | Unknown, Unknown |
| Huang et al | 96-well | Unknown, Unknown |

| Study | Solvent Used to Prepare PFAS Stock |
|-------|-----------------------------------|
| Vogs et al | DMSO |
| Menger et al | Unknown |
| Wang et al | Fish water |
| Gaballah et al | DMSO |
| Spulber et al | DMSO |
| Han et al | DMSO |
| Tu et al | Unknown |
| Huang et al | DMSO |
Figure S1. Model fits from the volume ($R^2 = 0.975$), embryo surface area ($R^2 = 0.998$), and hatching fraction models ($R^2 = 0.999$). For the surface area presentation, we show a comparison with a model that assumes purely spherical geometry for the embryo, and as mentioned previously, such an assumption underestimates surface area at longer times (greater embryo volume).
Figure S2a. Toxicokinetic model fits for both model forms considered presented on a linear mass scale. The kinetic form for the PFOA fit has the following metrics: $R^2 = 0.965$, RMSE = 1.37e-5 [µmol], degrees of freedom (DoF) = 16, AIC$_c$ = -381, and BIC = -379. The transporter form for the PFOA fit has the following metrics: $R^2 = 0.985$, RMSE = 8.86e-6 [µmol], DoF = 14, AIC$_c$ = -390, and BIC = -391. The kinetic form for the PFOS fit has the following metrics: $R^2 = 0.990$, RMSE = 1.84e-6 [µmol], DoF = 17, AIC$_c$ = -485, and BIC = -483. The transporter form for the PFOS fit has the following metrics: $R^2 = 0.993$, RMSE = 1.56e-6 [µmol], DoF = 15, AIC$_c$ = -484, and BIC = -484.
Figure S2b. Toxicokinetic model fits for both model forms considered presented on a log mass scale.
Figure S2c. The Vogs et al model fits, as found through our replication of their model by solving their equations and using their parameters. The fits are shown in the embryo concentration domain as done by Vogs et al. Due to the framework of the Vogs et al model and a lack of a chorion volume representation, a direct comparison with our proposed models in either the total system mass or embryo concentration domains is not possible. Thus, no information metrics (AICc and BIC) were calculated for comparative purposes. The following metrics for PFOA were calculated: $R^2 = 0.941$, RMSE = 36.6 [µM], and DoF = 2. The following metrics for PFOS were calculated: $R^2 = 0.997$, RMSE = 2.16 [µM], and DoF = 3.
Figure S3. (A) Net uptake data as directly reported by Vogs et al., assumed to be the numerical derivative of system mass data. (B) System concentration data taken from Vogs et al. and then subsequently converted to system mass, from which it was numerically differentiated to obtain net uptake. Vogs et al. claims that this data shows a biphasic uptake profile (i.e., pre- and post-hatching), but this is difficult to determine with the limited number of data points and noise of the data. A modeling argument needs to be made for biphasic uptake, which we present in Figure 2.
Figure S4. Transporter model results for a system mass comparison between a developing zebrafish that has its chorion intact and hatches naturally and one that has been dechorionated. The three different exposure concentrations of Vogs et al. are used for the analysis. A more noticeable difference is seen for PFOA over PFOS due to a greater relative mass flux across the chorion when compared to the embryo (dechorionated) for the PFOS case, rendering the chorion less of a mass transfer barrier for PFOS. Also, at the exposure concentrations for PFOS, the saturable nature of the transport for both the chorion and embryo is less relevant, but as the exposure concentration increases, the difference between the chorion and dechorionated cases increases.
Figure S5. A compartment-by-compartment analysis of PFAS mass for both models, which both indicate that the PFAS investigated have a higher affinity for the embryo over the chorion.
Modeling Code: Kinetic Form for PFOA

clear, clc, close all, format short g

% Volume model parameter input

rho_bar_0 = 1.70106925618241; % [unitless]
K_rho_bar = 0.00632224264296648; % [1/hr]
K_yolk = 0.00773377839001353; % [1/hr]
V_yolk_0 = 0.145281350592173e-6; % [mm^3] to [L]

% Declare the constant volume of the system (Kimmel et al 1995 chorion image analysis)

System_volume = ((4/3)*pi*(0.5^3))*10^-6; % [mm^3] to [L]

% Hatching model parameter input

n = 19.2526513155625; % [unitless]
K = 62.6340723966777; % [hr]

% Embryo volume to surface area parameter input

a = 12443072.3406685; % [mm^2/L]

% Chorion surface area

SA_C = 4*pi*(0.5^2); % [mm^2]

% Set parameters of the TK model

% Exposure concentration of PFOA

C_w = [21,41,340]; % [uM]

% Time interval

t_i = 2; % [hpf]
t_f = 120; % [hpf]

% Initial Conditions

C_chorion_0 = 0; % [uM]
C_embryo_0 = 0; % [uM]

% Data input

% Vogs et al 2019 raw data

Embryo_Time{1,1} = [24,48]; % [hr]
Embryo_Conc_Raw{1,1} = [15.3,68.9]; % [uM]
Embryo_Time{2,1} = [24,31,48]; % [hr]
Embryo_Conc_Raw{2,1} = [35,61.8,117.7]; % [uM]

Embryo_Time{3,1} = [3,6,9,24,31,48]; % [hr]
Embryo_Conc_Raw{3,1} = [36.5,28.4,43.4,100,165.1,298.7]; % [uM]

Embryo_Time_Full{1,1} = [24,48,72,96,120]; % [hr]
Embryo_Conc_Raw_Full{1,1} = [15.3,68.9,107.5,159.9,172.9]; % [uM]

Embryo_Time_Full{2,1} = [24,31,48,72,96,120]; % [hr]
Embryo_Conc_Raw_Full{2,1} = [35,61.8,117.7,157.4,229.6,258.8]; % [uM]

Embryo_Time_Full{3,1} = [3,6,9,24,31,48,72,96,120]; % [hr]
Embryo_Conc_Raw_Full{3,1} = [36.5,28.4,43.4,100,165.1,298.7,365.1,524.3,535]; % [uM]

% Convert the Vogs data to average mass/system Vogs growth model parameters

% Initial embryo volume
V_embryo_0 = 183.56e-9; % [nL] to [L]

% Embryo growth rate
g = 2.67e-9; % [nL/hr] to [L/hr]

for aa = 1:3
    Time_Vec = Embryo_Time{aa,1};
    Conc_Vec = Embryo_Conc_Raw{aa,1};

    Time_Vec_Full = Embryo_Time_Full{aa,1};
    Conc_Vec_Full = Embryo_Conc_Raw_Full{aa,1};

    Mass_Vec_Hold = [];
    Mass_Vec_Full_Hold = [];

    for bb = 1:length(Time_Vec)
        Time = Time_Vec(1,bb);
        Conc = Conc_Vec(1,bb);

        V_embryo_Brox = V_embryo_0+(g*Time); % [L/embryo]

        Mass_Vec_Hold(1,bb) = Conc*V_embryo_Brox; % [umol/system]
    end

    for bb = 1:length(Time_Vec_Full)
Time = Time_Vec_Full(1,bb);
Conc = Conc_Vec_Full(1,bb);

V_embryo_Brox = V_embryo_0+(g*Time); % [L/embryo]

Mass_Vec_Full_Hold(1,bb) = Conc*V_embryo_Brox; % [umol/system]
end

System_Mass{aa,1} = Mass_Vec_Hold;
System_Mass_Full{aa,1} = Mass_Vec_Full_Hold;
end

% Declare the fit parameter values
Fit_Param = [7.74455150133914e-07;1.21282514149277e-06;75.0513932459222;58.5093204061944];

% Plot all of the data with the model and also plot the fluxes
% Calculate the model
for aa = 1:3

% Calculate the concentrations

[t_calc,C_calc] = ode15s(@(t,C) dC_dt_Base_Model_2_4(t,C,C_w(aa),System_volume,Fit_Param(1),Fit_Param(2),Fit_Param(3),Fit_Param(4),rho_bar_0,K_rho_bar,K_yolk,V_yolk_0,n,K,S_A_C,a),[t_i:1:size(t_f)],[C_chorion_0,C_embryo_0]);
t_calc_hold{aa,1} = t_calc;
C_calc_hold{aa,1} = C_calc;

% Calculate the volumes
V_calc = f_Volume_Calc(t_calc, System_volume, rho_bar_0, K_rho_bar, K_yolk, V_yolk_0);
V_calc_hold{aa,1} = V_calc;

% Calculate the hatching fraction
HF = (t_calc.^n)./(((K^n)+(t_calc.^n)));

% Calculate the masses
M_chorion = (1-HF).*(C_calc(:,1).*V_calc(:,1)); % [umol]
M_embryo = C_calc(:,2).*(V_calc(:,2)+V_calc(:,3)); % [umol]

% Calculate the total system mass
Total_M_calc = M_chorion+M_embryo;
Total_M_calc_hold{aa,1} = Total_M_calc;

% Calculate the fluxes (only 340 uM)
if aa == 3

% Embryo surface area
SA_E = a.*(V_calc(:,2)+V_calc(:,3)); % [mm^2]

% Individual fluxes
Water_to_Chorion = (1-HF).*(Fit_Param(1)./(Fit_Param(3)+C_w(aa))).*C_w(aa).*SA_C;
Water_to_Embryo = HF.*(Fit_Param(2)./(Fit_Param(4)+C_w(aa))).*C_w(aa).*SA_E;
Chorion_to_Embryo = (1-HF).*(Fit_Param(2)./(Fit_Param(4)+C_calc(:,1))).*C_calc(:,1).*SA_E;

% Total chorion flux
chorion_flux = Water_to_Chorion-Chorion_to_Embryo;
% Total embryo flux
embryo_flux = Chorion_to_Embryo+Water_to_Embryo;
end
end

% Plot all of the data with the model

figure
plot(Embryo_Time_Full{1,1},System_Mass_Full{1,1},'ro',Embryo_Time_Full{2,1},System_Mass_Full{2,1},'bo',Embryo_Time_Full{3,1},System_Mass_Full{3,1},'go')
hold on
plot(t_calc_hold{1,1},Total_M_calc_hold{1,1},'r-',t_calc_hold{2,1},Total_M_calc_hold{2,1},'b-',t_calc_hold{3,1},Total_M_calc_hold{3,1},'g-')
xlabel('hpf')
ylabel('System PFOA Mass [\mumol]')
Legend_Vec{1,1} = '21 \muM Exposure';
Legend_Vec{2,1} = '41 \muM Exposure';
Legend_Vec{3,1} = '340 \muM Exposure';

legend(Legend_Vec,'Location','northwest');
Modeling Code: Kinetic Form for PFOS

clear, clc, close all, format short g

% Volume model parameter input
rho_bar_0 = 1.70106925618241; % [unitless]
K_rhoe_bar = 0.00632224264296648; % [1/hr]
K_yolk = 0.00773377839001353; % [1/hr]
V_yolk_0 = 0.145281350592173e-6; % [mm^3] to [L]

% Declare the constant volume of the system (Kimmel et al 1995 chorion image analysis)
System_volume = ((4/3)*pi*(0.5^3))*10^-6; % [mm^3] to [L]

% Hatching model parameter input
n = 19.2526513155625; % [unitless]
K = 62.6340723966777; % [hr]

% Embryo volume to surface area parameter input
a = 12443072.3406685; % [mm^2/L]

% Chorion surface area
SA_C = 4*pi*(0.5^2); % [mm^2]

% Set parameters of the TK model

% Exposure concentration of PFOS
C_w = [0.04,0.08,0.76]; % [uM]

% Time interval
t_i = 2; % [hpf]
t_f = 120; % [hpf]

% Initial Conditions
C_chorion_0 = 0; % [uM]
C_embryo_0 = 0; % [uM]

% Data input

% Vogs et al 2019 raw data
Embryo_Time{1,1} = [24,48]; % [hr]
Embryo_Conc_Raw{1,1} = [2.4,3.7]; % [uM]
Embryo_Time\{2,1\} = [9, 24, 31, 48]; \% [hr]
Embryo_Conc_Raw\{2,1\} = [1, 4.5, 5.4, 9.2]; \% [uM]

Embryo_Time\{3,1\} = [3, 6, 9, 24, 31, 48]; \% [hr]
Embryo_Conc_Raw\{3,1\} = [2, 3.2, 4.2, 14.8, 27.5, 47.2]; \% [uM]

Embryo_Time_Full\{1,1\} = [24, 48, 72, 96, 120]; \% [hr]
Embryo_Conc_Raw_Full\{1,1\} = [2.4, 3.7, 6.6, 8.4, 11.8]; \% [uM]

Embryo_Time_Full\{2,1\} = [9, 24, 31, 48, 72, 96, 120]; \% [hr]
Embryo_Conc_Raw_Full\{2,1\} = [1, 4.5, 5.4, 9.2, 14.7, 19, 23.3]; \% [uM]

Embryo_Time_Full\{3,1\} = [3, 6, 9, 24, 31, 48, 72, 96, 120]; \% [hr]
Embryo_Conc_Raw_Full\{3,1\} = [2, 3.2, 4.2, 14.8, 27.5, 47.2, 86.6, 114, 144.5]; \% [uM]

% Convert the Vogs data to average mass/system
% Vogs growth model parameters
%
% Initial embryo volume
V_embryo_0 = 183.56e-9; \% [nL] to [L]
%
% Embryo growth rate
g = 2.67e-9; \% [nL/hr] to [L/hr]

for \(aa = 1:3\)

Time_Vec = Embryo_Time\{aa,1\};
Conc_Vec = Embryo_Conc_Raw\{aa,1\};

Time_Vec_Full = Embryo_Time_Full\{aa,1\};
Conc_Vec_Full = Embryo_Conc_Raw_Full\{aa,1\};

Mass_Vec_Hold = [];
Mass_Vec_Full_Hold = [];

for \(bb = 1:length(Time_Vec)\)

Time = Time_Vec(1,bb);
Conc = Conc_Vec(1,bb);

V_embryo_Brox = V_embryo_0 + (g*Time); \% [L/embryo]

Mass_Vec_Hold(1,bb) = Conc*V_embryo_Brox; \% [umol/system]

end

for \(bb = 1:length(Time_Vec_Full)\)
Time = Time_Vec_Full(1,bb);  
Conc = Conc_Vec_Full(1,bb);

V_embryo_Brox = V_embryo_0+(g*Time);  % [L/embryo]

Mass_Vec_Full_Hold(1,bb) = Conc*V_embryo_Brox;  % [umol/system]

end

System_Mass{aa,1} = Mass_Vec_Hold;
System_Mass_Full{aa,1} = Mass_Vec_Full_Hold;

end

% Declare the fit parameter values

Fit_Param = [3.62877490179681e-07;6.44832794601536e-07;1.3077930104019;1.17098174154011];

% Plot all of the data with the model and calculate fluxes

% Calculate the model
for aa = 1:3

% Calculate the concentrations
[t_calc,C_calc] = ode15s(@(t,C)
dC_dt_Base_Model_2_4(t,C,C_w(aa),System_volume,Fit_Param(1),Fit_Param(2),Fit_Param(3),Fit_Param(4),rho_bar_0,K_rho_bar,K_yolk,V_yolk_0,n,K,SA_C,a),[t_i:1:t_f],[C_chorion_0,C_embryo_0]);
    t_calc_hold{aa,1} = t_calc;
    C_calc_hold{aa,1} = C_calc;

% Calculate the volumes
V_calc = f_Volume_Calc(t_calc,System_volume,rho_bar_0,K_rho_bar,K_yolk,V_yolk_0);
    V_calc_hold{aa,1} = V_calc;

% Calculate the hatching fraction
HF = (t_calc.^n)./(K^n+(t_calc.^n));

% Calculate the masses
M_chorion = (1-HF).*C_calc(:,1).*V_calc(:,1);  % [umol]
M_embryo = C_calc(:,2).*(V_calc(:,2)+V_calc(:,3));  % [umol]

% Calculate the total system mass

Total_M_calc = M_chorion+M_embryo;
Total_M_calc_hold{aa,1} = Total_M_calc;

% Calculate the fluxes (only 340 uM)
if aa == 3
    % Embryo surface area
    SA_E = a. *(V_calc(:,2)+V_calc(:,3)); % [mm^2]

    % Individual fluxes
    Water_to_Chorion = (1-HF). *(Fit_Param(1)./(Fit_Param(3)+C_w(aa))).*C_w(aa).*SA_C;
    Water_to_Embryo = HF. *(Fit_Param(2)./(Fit_Param(4)+C_w(aa))).*C_w(aa).*SA_E;
    Chorion_to_Embryo = (1-HF). *(Fit_Param(2)./(Fit_Param(4)+C_calc(:,1))).*C_calc(:,1).*SA_E;

    % Total chorion flux
    chorion_flux = Water_to_Chorion-Chorion_to_Embryo;

    % Total embryo flux
    embryo_flux = Chorion_to_Embryo+Water_to_Embryo;
end
end

% Plot all of the data with the model
figure
plot(Embryo_Time_Full{1,1},System_Mass_Full{1,1},'ro',Embryo_Time_Full{2,1},System_Mass_Full{2,1},'bo',Embryo_Time_Full{3,1},System_Mass_Full{3,1},'go')
hold on
    plot(t_calc_hold{1,1},Total_M_calc_hold{1,1},'r-',t_calc_hold{2,1},Total_M_calc_hold{2,1},'b-',t_calc_hold{3,1},Total_M_calc_hold{3,1},'g-')
xlabel('hpf')
ylabel('System PFOS Mass [\mumol]')
Legend_Vec{1,1} = '0.04 \mu M Exposure';
Legend_Vec{2,1} = '0.08 \mu M Exposure';
Legend_Vec{3,1} = '0.76 \mu M Exposure';

legend(Legend_Vec,'Location','northwest');
Modeling code: Functions for the Kinetic Form

```matlab
function dC =
    dC_dt_Base_Model_2_4(t,C,C_w,V_sys,k_wc,k_xe,K_sat_c,K_sat_e,rho_bar_0,K_rho_bar,K_yolk,V_yolk_0,n,K,SA_C,a)

    dC = zeros(2,1);

    % C_M(1) = chorion PFOA concentration [uM]
    % C_M(2) = embryo PFOA concentration (average individual) [uM]

    % Volumes
    V_yolk = V_yolk_0*exp(-K_yolk*t); % [L]
    V_embryo = exp(K_rho_bar*t)*(rho_bar_0*V_yolk_0*(-exp(-K_yolk*t)+1)); % [L]
    V_embryo = V_embryo+V_yolk; % [L]
    V_chorion = V_sys-V_embryo; % [L]

    dV_yolk = -K_yolk*V_yolk; % [L/hr]
    dV_embryo = (rho_bar_0*K_yolk*V_yolk_0*exp((K_rho_bar-K_yolk)*t))+(K_rho_bar*V_embryo); % [L/hr]
    dV_embryo = dV_embryo+dV_yolk; % [L/hr]
    dV_chorion = -dV_embryo; % [L/hr]

    % Hatching fraction
    HF = (t^n)/((K^n)+(t^n));

    % Embryo surface area
    SA_E = a*V_embryo; % [mm^2]

    % Differential Equations
    % Fluxes
    Water_to_Chorion = (k_wc/(K_sat_c+C_w))*C_w*SA_C;
    Water_to_Embryo = (k_xe/(K_sat_e+C_w))*C_w*SA_E;
    Chorion_to_Embryo = (k_xe/(K_sat_e+C(1)))*C(1)*SA_E;

    dC(1) = ((Water_to_Chorion-Chorion_to_Embryo)-(C(1)*dV_chorion))/V_chorion; % [uM/hr]
    dC(2) = (((1-HF)*Chorion_to_Embryo)+(HF*Water_to_Embryo))-(C(2)*dV_embryo))/V_embryo; % [uM/hr]
```
function Volume = f_Volume_Calc(t,V_sys,rho_bar_0,K_rho_bar,K_yolk,V_yolk_0)

% Volumes

V_yolk = V_yolk_0.*exp(-K_yolk.*t); % [L]
V_embryo = exp(K_rho_bar.*t).*(rho_bar_0.*V_yolk_0.*(-exp(-K_yolk.*t)+1)); % [L]
V_chorion = V_sys-V_yolk-V_embryo; % [L]

% Assign vectors to the matrix

Volume = [V_chorion,V_embryo,V_yolk];
Modeling Code: Transporter Form for PFOA

clear, clc, close all, format short g

% Volume model parameter input

rho_bar_0 = 1.70106925618241; % [unitless]
K_rho_bar = 0.00632224264296648; % [1/hr]
K_yolk = 0.00773377839001353; % [1/hr]
V_yolk_0 = 0.145281350592173e-6; % [mm^3] to [L]

% Declare the constant volume of the system (Kimmel et al 1995 chorion image analysis)

System_volume = ((4/3)*pi*(0.5^3))*10^-6; % [mm^3] to [L]

% Hatching model parameter input

n = 19.2526513155625; % [unitless]
K = 62.6340723966777; % [hr]

% Embryo volume to surface area parameter input

a = 12443072.3406685; % [mm^2/L]

% Chorion surface area

SA_C = 4*pi*(0.5^2); % [mm^2]

% Set parameters of the TK model

% Exposure concentration of PFOA

C_w = [21,41,340]; % [uM]

% Time interval

t_i = 2; % [hpf]
t_f = 120; % [hpf]

% Initial Conditions

C_chorion_0 = 0; % [uM]
M_chorion_bound_0 = 0; % [umol]

C_embryo_unhatched_0 = 0; % [uM]
M_embryo_unhatched_bound_0 = 0; % [umol]

C_embryo_average_0 = 0; % [uM]
M_embryo_average_bound_0 = 0; % [umol]

% Data input
% Vogs et al 2019 raw data

Embryo_Time{1,1} = [24,48]; % [hr]
Embryo_Conc_Raw{1,1} = [15.3,68.9]; % [uM]

Embryo_Time{2,1} = [24,31,48]; % [hr]
Embryo_Conc_Raw{2,1} = [35,61.8,117.7]; % [uM]

Embryo_Time{3,1} = [3,6,9,24,31,48]; % [hr]
Embryo_Conc_Raw{3,1} = [36.5,28.4,43.4,100,165.1,298.7]; % [uM]

Embryo_Time_Full{1,1} = [24,48,72,96,120]; % [hr]
Embryo_Conc_Raw_Full{1,1} = [15.3,68.9,107.5,159.9,172.9]; % [uM]

Embryo_Time_Full{2,1} = [24,31,48,72,96,120]; % [hr]
Embryo_Conc_Raw_Full{2,1} = [35,61.8,117.7,157.4,229.6,258.8]; % [uM]

Embryo_Time_Full{3,1} = [3,6,9,24,31,48,72,96,120]; % [hr]
Embryo_Conc_Raw_Full{3,1} = [36.5,28.4,43.4,100,165.1,298.7,365.1,524.3,535]; % [uM]

% Convert the Vogs data to average mass/system

% Vogs growth model parameters

% Initial embryo volume
V_embryo_0 = 183.56e-9; % [nL] to [L]

% Embryo growth rate

g = 2.67e-9; % [nL/hr] to [L/hr]

for aa = 1:3

    Time_Vec = Embryo_Time{aa,1};
    Conc_Vec = Embryo_Conc_Raw{aa,1};

    Time_Vec_Full = Embryo_Time_Full{aa,1};
    Conc_Vec_Full = Embryo_Conc_Raw_Full{aa,1};

    Mass_Vec_Hold = [];
    Mass_Vec_Full_Hold = [];

    for bb = 1:length(Time_Vec)

        Time = Time_Vec(1,bb);
        Conc = Conc_Vec(1,bb);

        V_embryo_Brox = V_embryo_0+(g*Time); % [L/embryo]

        Mass_Vec_Hold(1,bb) = Conc*V_embryo_Brox; % [umol/system]
end

for bb = 1:length(Time_Vec_Full)
    Time = Time_Vec_Full(1,bb);
    Conc = Conc_Vec_Full(1,bb);
    
    V_embryo_Brox = V_embryo_0+(g*Time); % [L/embryo]
    
    Mass_Vec_Full_Hold(1,bb) = Conc*V_embryo_Brox; % [umol/system]
end

System_Mass{aa,1} = Mass_Vec_Hold;
System_Mass_Full{aa,1} = Mass_Vec_Full_Hold;
end

% Declare the fit parameter values

Fit_Param = [0.000144072249273298;6.84224386609750e-05;3.44260205783938e-12;4.00271054753489e-06;144.187303041361;20.6919734998605];

% Plot all of the data with the model and also calculate fluxes

% Calculate the model

for aa = 1:3
    % Calculate the concentrations
    [t_calc,C_calc] = ode15s(@(t,C) dC_dt_Diffusion_Adsorption_Model_4_1(t,C,C_w(aa),System_volume,Fit_Param(1),Fit_Param(2),Fit_Param(3),Fit_Param(4),Fit_Param(5),Fit_Param(6),rho_bar_0,K_rho_bar,K_yolk,V_yolk_0,n,K,SA_C,a),[t_i:1:t_f],[C_chorion_0,M_chorion_bound_0,C_embryo_unhatched_0,M_embryo_unhatched_bound_0,C_embryo_average_0,M_embryo_average_bound_0]);
    t_calc_hold{aa,1} = t_calc;
    C_calc_hold{aa,1} = C_calc;

    % Calculate the volumes
    V_calc = f_Volume_Calc(t_calc,System_volume,rho_bar_0,K_rho_bar,K_yolk,V_yolk_0);
    V_calc_hold{aa,1} = V_calc;

    % Calculate the hatching fraction
    HF = (t_calc.^n)./(((K^n)+(t_calc.^n));

    % Calculate the masses
    M_chorion_free = (1-HF).*(C_calc(:,1).*V_calc(:,1)); % [umol]
M_chorion_bound = (1-HF).*C_calc(:,2); % [umol]
M_embryo_free = C_calc(:,5).*(V_calc(:,2)+V_calc(:,3)); % [umol]
M_embryo_bound = C_calc(:,6); % [umol]

% Calculate the total system mass
Total_M_calc = M_chorion_free+M_chorion_bound+M_embryo_free+M_embryo_bound;
Total_M_calc_hold{aa,1} = Total_M_calc;

% Calculate the total system volume
Total_V_calc = ((1-HF).*V_calc(:,1))+(V_calc(:,2)+V_calc(:,3));

% Calculate the total system concentration
Total_C_calc = Total_M_calc./Total_V_calc;

% Calculate the total system BCF
Total_BCF_calc = Total_C_calc./C_w(aa);
Total_BCF_calc_hold{aa,1} = Total_BCF_calc;

% Calculate the fluxes (only 340 uM)
if aa == 3
    % Embryo surface area
    SA_E = a.*(V_calc(:,2)+V_calc(:,3)); % [mm^2]

    % Individual fluxes
    Water_to_Chorion = (1-HF).*((Fit_Param(1))./(Fit_Param(5)+C_w(aa)).*(Fit_Param(5)+C_calc(:,1))).*(C_w(aa)-C_calc(:,1)).*SA_C;

    Water_to_Embryo = HP.*(Fit_Param(2))./(Fit_Param(6)+C_w(aa)).*(Fit_Param(6)+C_calc(:,5))).*(C_w(aa)-C_calc(:,5)).*SA_E;

    Chorion_to_Embryo_Average = (1-HF).*((Fit_Param(2))./(Fit_Param(6)+C_calc(:,1))).*(Fit_Param(6)+C_calc(:,5))).*(C_calc(:,1)-C_calc(:,5)).*SA_E;

    Chorion_to_Embryo_Unhatched = (1-HF).*((Fit_Param(2))./(Fit_Param(6)+C_calc(:,1))).*(Fit_Param(6)+C_calc(:,3))).*(C_calc(:,1)-C_calc(:,3)).*SA_E;

    % Bound fluxes
    dV_yolk = -K_yolk.*V_calc(:,3); % [L/hr]
\[ dV_{\text{embryo}} = (\rho_{\text{bar}} \cdot K_{\text{yolk}} \cdot V_{\text{yolk}} \cdot \exp((K_{\rho_{\text{bar}}} - K_{\text{yolk}}) \cdot t_{\text{calc}})) + (K_{\rho_{\text{bar}}} \cdot V_{\text{calc}}(:,2)); \quad [\text{L/hr}] \]

\[ dV_{\text{embryo}} = dV_{\text{embryo}} + dV_{\text{yolk}}; \quad [\text{L/hr}] \]

\[ dV_{\text{chorion}} = -dV_{\text{embryo}}; \quad [\text{L/hr}] \]

\[ \text{Chorion\_Bound} = (1-HF) \cdot \text{Fit\_Param}(3) \cdot (((\text{Water\_to\_Chorion} ./ (1-HF)) - (\text{Chorion\_to\_Embryo\_Unhatched} ./ (1-HF))) - (C_{\text{calc}}(:,1) \cdot dV_{\text{chorion}})) ./ (V_{\text{calc}}(:,1) + \text{Fit\_Param}(3)); \]

\[ \text{Embryo\_Bound} = \text{Fit\_Param}(4) \cdot (((\text{Chorion\_to\_Embryo\_Average} + \text{Water\_to\_Embryo}) - (C_{\text{calc}}(:,5) \cdot dV_{\text{embryo}})) ./ (V_{\text{calc}}(:,2) + V_{\text{calc}}(:,3) + \text{Fit\_Param}(4)); \]

% Total chorion flux
chorion_flux = Water_to_Chorion - Chorion_to_Embryo_Unhatched;

% Total embryo flux
embryo_flux = Chorion_to_Embryo_Average + Water_to_Embryo;

% Total system flux
total_flux = chorion_flux + embryo_flux;

end

end

% Plot all of the data with the model

figure

plot(Embryo_Time_Full{1,1},System_Mass_Full{1,1},'ro',Embryo_Time_Full{2,1},System_Mass_Full{2,1},'bo',Embryo_Time_Full{3,1},System_Mass_Full{3,1},'go')

hold on

plot(t_calc_hold{1,1},Total_M_calc_hold{1,1},'r-',t_calc_hold{2,1},Total_M_calc_hold{2,1},'b-',t_calc_hold{3,1},Total_M_calc_hold{3,1},'g-')

xlabel('

ylabel('System PFOA Mass [\text{\mu mol}]')

Legend_Vec{1,1} = '21 \text{\mu M Exposure}';
Legend_Vec{2,1} = '41 \text{\mu M Exposure}';
Legend_Vec{3,1} = '340 \text{\mu M Exposure}';
legend(Legend_Vec,'Location','northwest');

% Plot the mass as a function of time
C_calc_hold = C_calc_hold(3,1);
V_calc_hold = V_calc_hold(3,1);
Modeling Code: Transporter Form for PFOS

clear, clc, close all, format short g

% Volume model parameter input

\( \rho_{\text{bar}} = 1.70106925618241; \) [unitless]
\( K_{\rho_{\text{bar}}} = 0.00632224264296648; \) [1/hr]
\( K_{\text{yoik}} = 0.00773377839001353; \) [1/hr]
\( V_{\text{yoik}} = 0.145281350592173e^{-6}; \) [mm\(^3\) to L]

% Declare the constant volume of the system (Kimmel et al 1995 chorion image analysis)

\( \text{System\_volume} = ((4/3)*\pi*(0.5^3))*10^{-6}; \) [mm\(^3\) to L]

% Hatching model parameter input

\( n = 19.2526513155625; \) [unitless]
\( K = 62.6340723966777; \) [hr]

% Embryo volume to surface area parameter input

\( a = 12443072.3406685; \) [mm\(^2\)/L]

% Chorion surface area

\( SA_C = 4\pi*(0.5^2); \) [mm\(^2\)]

% Set parameters of the TK model

% Exposure concentration of PFOS

\( C_w = [0.04, 0.08, 0.76]; \) [uM]

% Time interval

\( t_i = 2; \) [hpf]
\( t_f = 120; \) [hpf]

% Initial Conditions

\( C_{\text{chorion}}_0 = 0; \) [uM]
\( M_{\text{chorion\_bound}}_0 = 0; \) [umol]

\( C_{\text{embryo\_unhatched}}_0 = 0; \) [uM]
\( M_{\text{embryo\_unhatched\_bound}}_0 = 0; \) [umol]

\( C_{\text{embryo\_average}}_0 = 0; \) [uM]
\( M_{\text{embryo\_average\_bound}}_0 = 0; \) [umol]

% Data input
% Vogs et al 2019 raw data

Embryo_Time{1,1} = [24,48]; % [hr]
Embryo_Conc_Raw{1,1} = [2.4,3.7]; % [uM]

Embryo_Time{2,1} = [9,24,31,48]; % [hr]
Embryo_Conc_Raw{2,1} = [1,4.5,5.4,9.2]; % [uM]

Embryo_Time{3,1} = [3,6,9,24,31,48]; % [hr]
Embryo_Conc_Raw{3,1} = [2,3.2,4.2,14.8,27.5,47.2]; % [uM]

Embryo_Time_Full{1,1} = [24,48,72,96,120]; % [hr]
Embryo_Conc_Raw_Full{1,1} = [2.4,3.7,6.6,8.4,11.8]; % [uM]

Embryo_Time_Full{2,1} = [9,24,31,48,72,96,120]; % [hr]
Embryo_Conc_Raw_Full{2,1} = [1,4.5,5.4,9.2,14.7,19,23.3]; % [uM]

Embryo_Time_Full{3,1} = [3,6,9,24,31,48,72,96,120]; % [hr]
Embryo_Conc_Raw_Full{3,1} = [2,3.2,4.2,14.8,27.5,47.2,86.6,114,144.5]; % [uM]

% Convert the Vogs data to average mass/system

% Vogs growth model parameters

% Initial embryo volume
V embargo_0 = 183.56e-9; % [nL] to [L]

% Embryo growth rate

for g = 2.67e-9; % [nL/hr] to [L/hr]

for aa = 1:3

Time_Vec = Embryo_Time{aa,1};
Conc_Vec = Embryo_Conc_Raw{aa,1};

Time_Vec_Full = Embryo_Time_Full{aa,1};
Conc_Vec_Full = Embryo_Conc_Raw_Full{aa,1};

Mass_Vec_Hold = [];
Mass_Vec_Full_Hold = [];

for bb = 1:length(Time_Vec)

Time = Time_Vec(1,bb);
Conc = Conc_Vec(1,bb);

V embargo_Brox = V embargo_0+g*Time; % [L/embryo]
Mass_Vec_Hold(1,bb) = Conc*V embargo_Brox; % [umol/system]
end

for bb = 1:length(Time_Vec_Full)
    Time = Time_Vec_Full(1,bb);
    Conc = Conc_Vec_Full(1,bb);

    V_embryo_Brox = V_embryo_0+(g*Time); % [L/embryo]
    Mass_Vec_Full_Hold(1,bb) = Conc*V_embryo_Brox; % [umol/system]
end

System_Mass{aa,1} = Mass_Vec_Hold;
System_Mass_Full{aa,1} = Mass_Vec_Full_Hold;
end

% Declare the fit parameter values
Fit_Param = [7.97460320063698e-06;5.59807186406287e-07;2.47089860217440e-09;0.108170700148128;3.28460593798294;1.05341917608744];

% Plot all of the data with the model and also calculate fluxes
% Calculate the model
for aa = 1:3
    % Calculate the concentrations
    [t_calc,C_calc] = ode15s(@(t,C) dC_dt_Diffusion_Adsorption_Model_4_1(t,C,C_w(aa),System_volume,Fit_Param(1),Fit_Param(2),Fit_Param(3),Fit_Param(4),Fit_Param(5),Fit_Param(6),rho_bar_0,K_rho_bar,K_yolk,V_yolk_0,n,K_SA,C,a),[t_i:1:t_f],[C_chorion_0,M_chorion_bound_0,C_embryo_unhatched_0,M_embryo_unhatched_bound_0,C_embryo_average_0,M_embryo_average_bound_0]);
    t_calc_hold{aa,1} = t_calc;
    C_calc_hold{aa,1} = C_calc;

    % Calculate the volumes
    V_calc = f_Volume_Calc(t_calc,System_volume,rho_bar_0,K_rho_bar,K_yolk,V_yolk_0);
    V_calc_hold{aa,1} = V_calc;

    % Calculate the hatching fraction
    HF = (t_calc.^n)./((K^n)+(t_calc.^n));

    % Calculate the masses
    M_chorion_free = (1-HF).*(C_calc(:,1).*V_calc(:,1)); % [umol]
\begin{verbatim}
M_chorion_bound = (1-HF).*C_calc(:,2);  \% [umol]
M_embryo_free = C_calc(:,5).*(V_calc(:,2)+V_calc(:,3));  \% [umol]
M_embryo_bound = C_calc(:,6);  \% [umol]

% Calculate the total system mass
Total_M_calc = M_chorion_free+M_chorion_bound+M_embryo_free+M_embryo_bound;
Total_M_calc_hold{aa,1} = Total_M_calc;

% Calculate the total system volume
Total_V_calc = ((1-HF).*V_calc(:,1))+(V_calc(:,2)+V_calc(:,3));

% Calculate the total system concentration
Total_C_calc = Total_M_calc./Total_V_calc;

% Calculate the total system BCF
Total_BCF_calc = Total_C_calc./C_w(aa);
Total_BCF_calc_hold{aa,1} = Total_BCF_calc;

% Calculate the fluxes (only 340 uM)
if aa == 3
  % Embryo surface area
  SA_E = a.*(V_calc(:,2)+V_calc(:,3));  \% [mm^2]

  % Individual fluxes
  Water_to_Chorion = (1-HF).*((Fit_Param(5)+C_w(aa)).*(Fit_Param(5)+C_calc(:,1)))).*(C_w(aa)-C_calc(:,1)).*SA_C;
  Water_to_Embryo = HF.*(Fit_Param(6).*(Fit_Param(6)+C_w(aa)).*(Fit_Param(6)+C_calc(:,5)))).*(C_w(aa)-C_calc(:,5)).*SA_E;
  Chorion_to_Embryo_Average = (1-HF).*((Fit_Param(5)+C_calc(:,1)).*(Fit_Param(5)+C_calc(:,5)))).*(C_calc(:,1)-C_calc(:,5)).*SA_E;
  Chorion_to_Embryo_Unhatched = (1-HF).*((Fit_Param(5)+C_calc(:,1)).*(Fit_Param(5)+C_calc(:,3)))).*(C_calc(:,1)-C_calc(:,3)).*SA_E;

  % Bound fluxes
  dV_yolk = -K_yolk.*V_calc(:,3);  \% [L/hr]
\end{verbatim}
dV_embryo = (rho_bar_0.*K_yolk.*V_yolk_0.*exp((K_rho_bar-K_yolk).*t_calc))+(K_rho_bar.*V_calc(:,2)); % [L/hr]

dV_embryo = dV_embryo+dV_yolk; % [L/hr]

dV_chorion = -dV_embryo; % [L/hr]

Chorion_Bound = (1-HF).*Fit_Param(3).*(((Water_to_Chorion./(1-HF))-(Chorion_to_Embryo_Unhatched./((1-HF)))-(C_calc(:,1).*dV_chorion))./(V_calc(:,1)+Fit_Param(3)));

Embryo_Bound = Fit_Param(4).*(((Chorion_to_Embryo_Average+Water_to_Embryo)-(C_calc(:,5).*dV_embryo))./(V_calc(:,2)+V_calc(:,3)+Fit_Param(4)));

chorion_flux = Water_to_Chorion-Chorion_to_Embryo_Unhatched;

embryo_flux = Chorion_to_Embryo_Average+Water_to_Embryo;

total_flux = chorion_flux+embryo_flux;

ej
end

end

% Plot all of the data with the model

figure

plot(Embryo_Time_Full{1,1},System_Mass_Full{1,1},'ro',Embryo_Time_Full{2,1},System_Mass_Full{2,1},'bo',Embryo_Time_Full{3,1},System_Mass_Full{3,1},'go')

hold on

plot(t_calc_hold{1,1},Total_M_calc_hold{1,1},'r-',t_calc_hold{2,1},Total_M_calc_hold{2,1},'b-',t_calc_hold{3,1},Total_M_calc_hold{3,1},'g-')

xlabel('hpf')
ylabel('System PFOS Mass [\mumol]')

Legend_Vec{1,1} = '0.04 \mumM Exposure';
Legend_Vec{2,1} = '0.08 \mumM Exposure';
Legend_Vec{3,1} = '0.76 \mumM Exposure';
legend(Legend_Vec,'Location','northwest');
Modeling code: Functions for the Transporter Form

```matlab
function dC = dC_dt_Diffusion_Adsorption_Model_4_1(t,C,C_w,V_sys,k_wc,k_xe,K_ads_c,K_ads_e,K_sat_c,K_sat_e,rho_bar_0,K_rho_bar,K_yolk,V_yolk_0,n,K,SA_C,a)

dC = zeros(6,1);

% C(1) = chorion free PFOA concentration [uM]
% C(2) = chorion bound PFOA mass [umol]
% C(3) = embryo free PFOA concentration (unhatched) [uM]
% C(4) = embryo bound PFOA mass (unhatched) [umol]
% C(5) = embryo free PFOA concentration (average) [uM]
% C(6) = embryo bound PFOA mass (average) [umol]

% Volumes
V_yolk = V_yolk_0*exp(-K_yolk*t); % [L]
V_embryo = exp(K_rho_bar*t)*(rho_bar_0*V_yolk_0*(-exp(-K_yolk*t))+1); % [L]
V_embryo = V_embryo+V_yolk; % [L]
V_chorion = V_sys-V_embryo; % [L]

dV_yolk = -K_yolk*V_yolk; % [L/hr]
dV_embryo = (rho_bar_0*K_yolk*V_yolk_0*exp((K_rho_bar-K_yolk)*t))+(K_rho_bar*V_embryo); % [L/hr]
dV_embryo = dV_embryo+dV_yolk; % [L/hr]

% Hatching fraction
HF = (t^n)/((K^n)+(t^n));

% Embryo surface area
SA_E = a*V_embryo; % [mm^2]

% Differential Equations

% Fluxes
Water_to_Chorion = (k_wc/((K_sat_c+C_w)*(K_sat_c+C(1))))*(C_w-C(1))*SA_C;
Water_to_Embryo = (k_xe/((K_sat_e+C_w)*(K_sat_e+C(5))))*(C_w-C(5))*SA_E;

Chorion_to_Embryo_Unhatched = (k_xe/((K_sat_e+C(1))*(K_sat_e+C(3))))*(C(1)-C(3))*SA_E;
Chorion_to_Embryo_Average = (k_xe/((K_sat_e+C(1))*(K_sat_e+C(5))))*(C(1)-C(5))*SA_E;
```

\[ dC(1) = \left(\frac{\text{Water to Chorion-Chorion to Embryo Unhatched}}{V_{\text{chorion}}+K_{ads_c}}\right) \times \left(\frac{dV_{\text{chorion}}}{t}\right); \quad \text{[uM/hr]} \]

\[ dC(2) = K_{ads_c} \times dC(1); \quad \text{[umol/hr]} \]

\[ dC(3) = \left(\frac{\text{Chorion to Embryo Unhatched}}{V_{\text{embryo}}+K_{ads_e}}\right) \times \left(\frac{dV_{\text{embryo}}}{t}\right); \quad \text{[uM/hr]} \]

\[ dC(4) = K_{ads_e} \times dC(3); \quad \text{[umol/hr]} \]

\[ dC(5) = \left(\frac{\text{Chorion to Embryo Average}+(HF \times \text{Water to Embryo})}{V_{\text{embryo}}+K_{ads_e}}\right) \times \left(\frac{dV_{\text{embryo}}}{t}\right); \quad \text{[uM/hr]} \]

\[ dC(6) = K_{ads_e} \times dC(5); \quad \text{[umol/hr]} \]

---

**function** Volume = f_Volume_Calc\( (t, V_{sys}, \rho_{bar_0}, \rho_{bar}, K_{yolk}, V_{yolk_0}) \)

% Volumes

\[
V_{yolk} = V_{yolk_0} \times \exp(-K_{yolk} \times t); \quad \text{[L]}
\]

\[
V_{embryo} = \exp(K_{rho_bar} \times t) \times (\rho_{bar_0} \times V_{yolk_0} \times (\exp(-K_{yolk} \times t)+1)); \quad \text{[L]}
\]

\[
V_{chorion} = V_{sys} - V_{yolk} - V_{embryo}; \quad \text{[L]}
\]

% Assign vectors to the matrix

Volume = [V_{chorion}, V_{embryo}, V_{yolk}];
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