SUPPORTING INFORMATION
for the paper

Bioconcentration of several series of cationic surfactants in rainbow trout

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**Text S1: Quality assurance of the analytical methods**

For the water method, the repeatability, quantified as the average relative standard deviation of the 21 sets of triplicate samples collected during the exposure phase, ranged between 1-6% (Table S6). Outliers were rare, accounting for at most 6% of the measurements, and were with one exception positive outliers that impacted several or all of the more hydrophobic analytes in a given sample. We believe that the outliers were caused by the presence of a large particle (e.g., a feces fragment) in the sampled water, and these data were therefore discarded. The concentrations in the method blanks for the water samples were low (Table S7), with the maximum blank seldom exceeding 1% of the target concentration in water.

For fish analysis, method precision was assessed by analyzing inter-batch duplicates for a selection of the samples. When the replication only included sample clean-up and instrumental analysis (i.e., a common extract was used), the mean relative difference between the duplicates ranged from 2% to 7% when the concentrations were above the LOQ (Table S8). When the replication also included the extraction step, the mean relative difference was similar (Table S9). The mean recovery of standards spiked to fish homogenate or raw extract ranged from 92-116% (see Table S10), which provided confidence in the accuracy of the method. Experiments were conducted to test extraction efficiency, and the extracted quantity of test chemical decreased by a factor of 10 between the first and second, and again between the second and third 1 h batch sonication steps (see Figure S1). This indicated that 2 batch extractions were sufficiently exhaustive, and this procedure was chosen.

The test chemical concentrations in the control fish and the fish method blanks were <5 ng g\(^{-1}\) in most cases. The LOQ of the method (calculated as 10 × standard deviation of the blank/control fish) ranged from 3-9 ng g\(^{-1}\) (Table S11). However, two batches for fish from the exposure phase of the MIX1pH experiment had elevated blank levels of P9, T10 and T13 (39±5, 202±21 and 59±2 ng g\(^{-1}\), respectively). The resulting blank correction resulted in P9 being unquantifiable for this experiment and T10 lying between the LOD and LOQ for 16 of 24 data points during the exposure phase. The data for T10 were used for model fitting despite their higher uncertainty.
Text S2: Model fitting

The data were fit by integrating the equation

\[ \frac{dC_F}{dt} = k_U C_W - k_T C_F \]

stepwise from one sampling time point to the next. A function of concentration in water versus time was created from the measurements for both the exposure phase and elimination phase, so no information was lost by averaging the concentrations over the exposure phase or assuming a concentration of zero during elimination. The concentration in water \( C_W \) was assumed to be constant between sampling time points and equal to the average of the concentrations measured at the start and the end of the interval (see Tables S14-S16 for the measured data). The concentration in fish at the end of the interval \( C_{F(t+1)} \) could be then calculated from the concentration at the beginning of the interval \( C_{F(t)} \) using

\[ C_{F(t+1)} = \left( C_{F(t)} - \frac{k_U}{k_T} C_W \right) e^{-(k_T \Delta t)} + \frac{k_U}{k_T} C_W \]

The model was fitted simultaneously for \( k_U \) and \( k_T \) using the solver function in Microsoft Excel. The fitting criterion was to minimize the sum of the normalized residuals \(|C_{F(measured)} - C_{F(modelled)}|\), whereby the normalization was done to the smaller of the measured and modeled concentrations:

\[ \text{fitting criterion} = \min \sum \left| \frac{C_{F(measured)} - C_{F(modelled)}}{\min(C_{F(measured)}, C_{F(modelled)})} \right| \]

By normalizing the residuals, the bias associated with the magnitude of the concentration is eliminated. The fitting was done using \( C_{F(measured)} \) for individual fish. All time points were included for which all three fish were above the LOQ, with the exception of the first 3 time points during the exposure phase. They were not included in the fitting procedure as there was evidence of a small, rapidly responding compartment that led to large relative errors at the beginning of the model simulation, errors which then dominated the fitting criterion. This small, rapidly responding compartment may have been skin mucus (Kierkegaard et al., 2020). For P12 in the MIX1pH experiment, three fish were removed as outliers; these fish had concentrations that were an order of magnitude higher than the others.
**Text S3: A mechanistic model of amine bioconcentration**

The one-box mass balance model of chemical bioconcentration in fish considers three mass transfer processes: diffusive uptake across the gills, diffusive elimination across the gills, and biotransformation (Barber, 2003). The governing mass balance equation is:

\[
M \frac{dC_F}{dt} = \frac{1}{R_{W-B}} A_G C_W - \frac{1}{R_{B-W}} D_{FW} C_F - M k_B C_F
\]  

where \( M \) is fish mass (kg), \( A_G \) is the surface area of the gills (m\(^2\)), \( C_F \) is concentration of the chemical in the fish (mol kg\(^{-1}\)), \( C_W \) is the concentration of freely dissolved chemical in the water (mol m\(^{-3}\)), \( D_{FW} \) is the distribution ratio of the chemical (both charged and neutral forms) between the fish and water (m\(^3\) kg\(^{-1}\)), \( R_{W-B} \) and \( R_{B-W} \) are the overall resistances for transport across the gills from water to blood and blood to water, respectively (h m\(^{-1}\)), and \( k_B \) is the first order rate constant for transformation of the chemical in the fish (h\(^{-1}\)) (Erickson et al., 2006). Comparing with the rate constant model used for bioconcentration assessment (OECD, 2012)

\[
\frac{dC_F}{dt} = k_U C_W - k_T C_F
\]

the uptake rate constant \( k_U \) is defined as:

\[
k_U = \frac{1000 A_G}{R_{W-B} M}
\]

where 1000 is a conversion factor from m\(^3\) to L, and the elimination rate constant \( k_T \) is defined as:

\[
k_T = \frac{1}{R_{B-W} M D_{FW}} + k_B = k_2 + k_B
\]

where \( k_2 \) is the rate constant for gill elimination. \( M \) was set to the average mass of the fish in the experiment (0.01094 kg). \( A_G \) was calculated for a fish of this mass using the correlation for rainbow trout in Hughes (1984). \( D_{FW} \) was calculated as 1% of the membrane-water distribution coefficient (\( D_{MLW,T} \), m\(^2\) kg\(^{-1}\)), since fish contain approximately 1% membrane lipids by mass (Hendriks et al., 2005). \( D_{MLW,T} \) was based on measurements of the distribution ratio between water and a neutral phospholipid experimental model (\( D_{MLW} \), m\(^2\) kg\(^{-1}\), see Table S3). The selection of \( D_{MLW,T} \) as the basis for \( D_{FW} \) was based on:

(i) the availability of sufficient \( D_{MLW} \) values to allow for extrapolations to all test compounds (Timmer and Droge, 2017) and insufficient data on other tissue components;
(ii) measurements showing for one cationic surfactant that partitioning to membrane lipids is greater than to structural muscle protein (\( D_{SPW} \)) (Cs-benzalkonium log \( D_{MLW} \) is 3.11-3.63; (Timmer and Droge, 2017, Bittermann et al., 2014), log \( D_{SPW} \) is 1.4 (Henneberger et al., 2016));
(iii) \( D_{SPW} \) for several cations being comparable to albumin blood protein (\( D_{BPW} \)) (Henneberger et al., 2016).

Membranes also contain anionic phospholipids which enhance the sorption of cations (Elsayed et al., 2009). This effect was accounted for using a regression developed by Schmitt:

\[
D_{MLW,T} = D_{MLW} (1 + 20 f_{PL-})
\]

where \( f_{PL-} \) is the fraction of anionic phospholipids in the membrane. \( f_{PL-} \) was set to 0.175 based on data reported in Schmitt (2008).
$R_{W\rightarrow B}$ and $R_{B\rightarrow W}$ can be treated as the sum of 4 resistances acting in series: water ($R_W$), membrane ($R_M$), cytosol ($R_C$) and blood ($R_B$). The water and blood flow resistances were assumed to be flow governed and estimated as

$$R_{W, W\rightarrow B} = \frac{A_G}{Q_W}$$  \hspace{.5cm} (S4.6)

$$R_{W, B\rightarrow W} = \frac{A_G}{Q_W} \frac{\alpha_W}{\alpha_B}$$  \hspace{.5cm} (S4.7)

$$R_{B, W\rightarrow B} = \frac{A_G}{Q_B(1 + f_{PL-B}D_{MLW})} \frac{\alpha_B}{\alpha_W}$$  \hspace{.5cm} (S4.8)

$$R_{B, B\rightarrow W} = \frac{A_G}{Q_B(1 + f_{PL-B}D_{MLW})}$$  \hspace{.5cm} (S4.9)

where $Q_W$ and $Q_B$ are the flow rates through the gills of water and blood, respectively ($\text{m}^3\text{ h}^{-1}$), $f_{PL-B}$ is the fraction of polar lipids in the blood, and $\alpha_W$ and $\alpha_B$ are the neutral fraction of the chemical in the water and blood, respectively (Erickson et al., 2006). The extra term in the denominator of the blood resistance equations accounts for the increased transport capacity due to sorption of the chemicals to membrane lipids present in the blood. No influence of sorption on transport capacity was included for water. The quotient of the neutral fractions is a consequence of only the neutral fraction of the chemical passing through the membrane. It accounts for the change in the transport capacity of the distant medium with respect to the reference medium (water in the case of $R_{W\rightarrow B}$ and blood in the case of $R_{B\rightarrow W}$) as a result of the change in pH.

We did not find sufficient information to construct a well-constrained model of the resistances for membrane ($R_M$), and cytosol ($R_C$) for cationic surfactants. They are unknown in the model.

The model was parameterized for the conditions used in the experiment. $Q_W$ was estimated to be $2.80 \times 10^{-4}$ $\text{m}^3\text{ h}^{-1}$ based on an average fish mass of 10.9 g, water temperature of 10 °C and an $O_2$ saturation of 95% using the method of Arnot and Gobas (2004). $Q_B$ was $2.45 \times 10^{-5}$ $\text{m}^3\text{ h}^{-1}$ using the method of Erickson et al. (2006). $f_{PL-B}$ was set equal to 0.007, 50% of the lipid of rainbow trout blood reported by Bertelsen et al. (1998). The pH of water at the gill surface, and hence $\alpha_W$, varied along the gill lamella (see Text S5). Therefore, the gill was treated as 10 sequential segments. The resistances were determined for each segment using the water pH in that segment (see Text S5). These 10 parallel resistances were then combined to give an overall resistance.

The water flow and blood flow resistances for elimination each differ from the corresponding resistance for uptake by a constant, the ratio of the neutral fraction of the chemical in water and blood $\alpha_W/\alpha_B$ (Eq. S4.7 and S4.8). This is also true for membrane and cytosol resistances when the uptake of the charged form is negligible (Erickson et al., 2006). Consequently, the relative contributions of the different resistances are the same during uptake and elimination, and the overall resistance for gill elimination $R_{B\rightarrow W}$ can be calculated from the overall resistance for gill uptake $R_{W\rightarrow B}$:

$$R_{B\rightarrow W} = \frac{\alpha_W}{\alpha_B} R_{W\rightarrow B}$$  \hspace{.5cm} (S4.10)

where $\alpha_W/\alpha_B$ describes the effect of ion trapping on the resistance. Substituting into Eq. 4.4, it follows that the elimination rate constant can be defined as follows:

$$k_2 = \frac{\alpha_B k_\beta}{\alpha_W D_{FW}}$$  \hspace{.5cm} (S4.11)
**Text S4:** Estimation of the fraction of the surfactants in aquarium water during the exposure experiment that was sorbed to organic material

The organic carbon-water distribution coefficient $D_{OC} \text{[L kg}^{-1}\text{]}$ was estimated with a model derived from a large set of measurements of distribution of cationic surfactants between water and standard soil organic matter in medium with a divalent cation content of 5 mM Ca$^{2+}$ (Droge and Goss, 2013):

$$
\log D_{OC} = 1.70 V_x + 0.397 N A_i - 0.725
$$

where $V_x$ is McGowan’s molecular volume and $N A_i$ is the number of H-atoms attached to the charged N moiety.

The $D_{OC}$ value for protonated amines and QAC was used together with the average TOC concentration in the aquaria (5.5 mg L$^{-1}$) to estimate the freely dissolved fraction. The results are summarized in this table, with freely dissolved fractions calculated (with TOC in units of kg L$^{-1}$):

$$
\text{Freely dissolved fraction} = \frac{1}{1 + (D_{OC} \cdot TOC)}
$$

| Chemical | $V_x$  | $N A_i$ | $\log D_{OC}$ | Freely dissolved fractions |
|----------|--------|---------|----------------|---------------------------|
| P9       | 1.4765 | 3       | 2.98           | 0.99                      |
| P12      | 1.9207 | 3       | 3.73           | 0.97                      |
| P13      | 2.0401 | 3       | 3.93           | 0.96                      |
| P16      | 2.4628 | 3       | 4.65           | 0.80                      |
| S12      | 2.0401 | 2       | 3.54           | 0.98                      |
| S16      | 2.6037 | 2       | 4.50           | 0.85                      |
| T9       | 1.7583 | 2       | 3.06           | 0.99                      |
| T10      | 1.8992 | 1       | 2.90           | 1.00                      |
| T13      | 2.3219 | 1       | 3.62           | 0.98                      |
| T14      | 2.4628 | 1       | 3.86           | 0.96                      |
| Q10      | 2.0831 | 0       | 2.82           | 1.00                      |
| Q14      | 2.6467 | 0       | 3.77           | 0.97                      |

The freely dissolved fraction was smallest for P16, for which 80% of the amount in water was estimated to be freely dissolved.

At a 10 times lower water hardness than anticipated by 5 mM Ca$^{2+}$, which may be more reflective of the aquarium water, the $\log D_{OC}$ is expected to increase by 0.3 log units. For the strongest sorbing amine P16 an estimated $\log D_{OC}$ (at 0.5 mM Ca$^{2+}$) of 4.95, this could result in a further reduction of the freely dissolved fraction to 0.67. The apparent aqueous concentration of P16 to which the fish were exposed may thus be overestimated by 33%, which would increase the fitted BCF proportionally.
**Text S5: Estimation of pH at the gill surface**

The pH at the surface of the gills was calculated using the following system of 5 equations:

\[
\frac{[H^+][HCO_3^−]}{[H_2CO_3]} = K_1
\]

\[
\frac{[H^+][CO_3^{2−}]}{[HCO_3^−]} = K_2
\]

\[
alk = 2 \times \left[CO_3^{2−}\right] + \left[HCO_3^-\right]
\]

\[
DIC = \left[H_2CO_3\right] + \left[HCO_3^-\right] + \left[CO_3^{2−}\right]
\]

\[
pH = −\log([H^+])
\]

where alk is alkalinity and DIC is total dissolved inorganic carbon. The dissociation constants \(K_1\) and \(K_2\) were estimated from water temperature according to equations 9.23 and 9.24 in

[http://www-naweb.iaea.org/napc/ih/documents/global_cycle/vol%20I/cht_i_09.pdf](http://www-naweb.iaea.org/napc/ih/documents/global_cycle/vol%20I/cht_i_09.pdf)

The measured pH and alkalinity in bulk water were used to solve for \([H^+], [HCO_3^-], [CO_3^{2−}], [H_2CO_3]\) and DIC. For the water in the gill lamella, the carbon added to the water via respiration (0.0002 mol L\(^{-1}\) based on estimated oxygen demand and gill ventilation rate for a 10 g fish at 10 °C (Arnot et al. 2008, Armitage et al. 2013)) was added to DIC, and using this new DIC and the alkalinity the 5 equations were resolved for \([H^+], [HCO_3^-], [CO_3^{2−}], [H_2CO_3]\) and pH. The pH gradient along the lamella was approximated by dividing the lamella into 10 segments and assuming that the CO\(_2\) excretion rate was uniform along the length of the lamella. The resulting gradient of pH along the lamella is shown below.
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| TONNAGE (y^-1) | CAS         | Amine Type | Chain length range (*“average chain length”*) | SUBSTANCE NAME                                                                 | SURFACE TENSION | REACH Dossier # | Examples on BCF info in REACH-dossier |
|---------------|-------------|------------|-----------------------------------------------|-------------------------------------------------------------------------------|-----------------|-----------------|--------------------------------------|
| 1000000-1000000 | 1335202-88-4 | Q          | C16-18 (17)                                   | Esterification products of fatty acids, C16-18 (even numbered) and C18 (unsaturated) with triethanolamine, dimethyl sulphate-quaternized | 39.4            | 15243           | Reference to in vivo BCF: DODMAC      |
| 100000-100000   | 68037-95-6   | P          | C16-18 (17)                                   | Amines, C16-18 and C18-unsatd. Alkyl                                          | 23.1            | 14682           |                                      |
| 100000-100000   | 90640-32-7   | P          | C16-18 (17)                                   | Amines, C16-18-alkyl                                                           | 23              | 14440           |                                      |
| 100000-100000   | 68037-95-6   | P          | C16-18 (17)                                   | Amines, C16-18 and C18-unsatd. Alkyl                                          | 23.9            | 14682           |                                      |
| 100000-100000   | 90640-32-7   | P          | C16-18 (17)                                   | Amines, C16-18-alkyl                                                           | 51.3            | 14440           |                                      |
| 100000-100000   | 1079184-43-2 | Q          | C16-18 (17)                                   | Ethanaminium, 2-hydroxy-N-(2-hydroxyethyl)-N,N-dimethyl-, esters (C16-18/C18-unsatd. fatty ac) Cl | 37.5            | 5845            | Reference to in vivo BCF: DODMAC      |
| 100000-100000   | 1079184-43-2 | Q          | C16-18 (17)                                   | Ethanaminium, 2-hydroxy-N-(2-hydroxyethyl)-N,N-dimethyl-, esters (C16-18/C18-unsatd. fatty ac) Cl | <40             | 5845            |                                      |
| 1000-100000     | No CAS      | P          | C9-11 iso (10)                                | 3-((C9-11-iso,C10-rich)alkyloxy)propan-1-amine                                 | 30              | 12430           |                                      |
| 1000-100000     | 124-22-1     | P          | C12                                          | dodecan-1-amine                                                               | 23.1            | 15915           |                                      |
| 1000-100000     | 111-86-4     | P          | C8                                           | octan-1-amine                                                                | 44.2            | 1996            |                                      |
| 1000-100000     | 124-30-1     | P          | C18                                          | octadecan-1-amine                                                            | 23.9            | 14418           |                                      |
| 1000-100000     | 2156592-58-2 | P          | C12-18 (15)                                   | Amines, C12-18-(even numbered) and C18-(unsaturated) alkyl                   | 24.3            | 19387           |                                      |
| 1000-100000     | 1213789-63-9 | P          | C16-18 (17)                                   | (Z)-octadec-9-enylamine, C16-18-(even numbered, saturated and unsaturated)-alkylamines | 24.3            | 16132           |                                      |
| 1000-100000     | 1226892-43-8 | P          | C18                                          | Fatty acids, C18 (unsaturated), reaction products with diethylenetriamine   | 34              | 15906           |                                      |
| 1000-100000     | 1213789-63-9 | P          | C18                                          | (Z)-octadec-9-enylamine, C16-18-(even numbered, saturated and unsaturated)-alkylamines | 51.3            | 16132           |                                      |
| 1000-100000     | 111-92-2     | S          | C4x2 (8)                                     | N-butybutan-1-amine                                                          | 50.6            | 13527           |                                      |
| 1000-100000     | 84649-84-3   | T          | C12-14 (13)                                   | Amines, C12-14-alkyldimethyl                                               | 25.2            | 14216           | BCF data waiving: study scientifically not necessary / other information available |
| 1000-100000     | 68391-04-8   | T          | C12-18 (15)                                   | Amines, C12-18-alkyldimethyl                                               | 30.5            | 13558           |                                      |
| 1000-100000     | 112-69-6     | T          | C16                                          | N,N-dimethylethadecan-1-amine                                               | 30.5            | 14932           |                                      |
| 1000-100000     | 124-28-7     | T          | C18                                          | dimethyl(octadecyl)amine                                                    | 32.2            | 14746           |                                      |
| 1000+           | 71786-60-2   | T          | C12-18 DEA (15)                               | Ethanol, 2,2’-iminobis-, N-C12-18-alkyl derivs.                               | 29              | 14237           | BCF estimated by Kow + in vitro S9, with measured log Kow of 0.7               |
| 1000+           | 25307-17-9   | T          | C18 DEA                                      | 2-[(2-hydroxyethyl)[(9E)-octadec-9-en-1-yl]amino]ethan-1-ol                   | 27              | 14853           | BCF estimated by BCFBAF-model, with measured log Kow of 3.6                   |
| 1000+           | 1218787-30-4 | T          | C16-18 DEA (17)                               | unnamed 2,2’-(C16-18 (even numbered) alkyl imino) diethanol                 | 30              | 14327           | BCF estimated by BCFBAF-model, with measured log Kow of 3.6                   |
| Tonnage | CAS Number | Structure | CAS Number of Reference | Reference to in vivo BCF | Applicability |
|---------|------------|-----------|-------------------------|--------------------------|---------------|
| 1000+   | 1218787-32-6 | C18 DEA 2,2’-(C16-18 (even numbered, C18 unsaturated) alkyl imino) diethanol | 2705260-06-3 | | BCF estimated by BCFBAF-model. Test substance was found to fall in the applicability domain |
| 1000-10000 | 68424-85-1 | C12-16 BAC (14) Quaternary ammonium compounds, benzyl C12-16 (even numbered)-alkyldimethyl chlorides | 2705260-06-3 | | |
| 1000-10000 | 68391-05-9 | C14x2 (28) N,N-dimethyl-N-tetradecyltetradecan-1-aminium chloride | 2705260-06-3 | | |
| 1000+   | No CAS | C16-18 PO (17) 1-Propanaminium, 2-hydroxy-N-(2-hydroxypropyl)-N,N-dimethyl-, esters with fatty acids, C16-18 (even numbered) and C18 unsaturated., Me sulfates (salts) | 2705260-06-3 | | Reference to in vivo BCF: DODMAC |
| 1000-10000 | 1125503-33-4 | C12-14 EO (13) N-(2-hydroxyethyl)-N,N-dimethyl alkyl-C12-14-(even numbered)-1-aminium chloride | 2705260-06-3 | | |
| 1000-10000 | 68909-18-2 | C7 Pyridinium, 1-(phenylmethyl)-, ethyl methyl derivs., chlorides | 2705260-06-3 | | |

**Lower tonnage cations of interest**

| Tonnage | CAS Number | Structure | CAS Number of Reference | Reference to in vivo BCF |
|---------|------------|-----------|-------------------------|--------------------------|
| 100-1000 | 139-08-2 | C14 BAC benzyl(dimethyl)tetradecylazanium chloride | 2705260-06-3 | Reference to in vivo BCF: C12-16-ADBAC |
| 100-1000 | 68391-03-7 | C12-18 (15) Quaternary ammonium compounds, C12-C18 (even numbered) alkyltrimethyl chloride | 2705260-06-3 | Reference to in vivo BCF: C12-16-ADBAC |
| 100-1000 | 1273322-47-6 | C16-18 (17) C16-18-(even numbered, C18-unsaturated)-alkylamines acetates | 2705260-06-3 | Reference to in vivo BCF: C12-16-ADBAC |
| 100-1000 | 94667-33-1 | C14x2 EO (28) (Bardap 26) Poly(oxy-1,2-ethanediyl), alpha-[(didecylmethylammonio)ethyl] omega-hydroxy-2-propionate (salt) | 2705260-06-3 | Reference to in vivo BCF: DDAC/Bardac22 |
| 0-10 | 210420-85-2 | C10x2 (20) 1-Decanaminium,N-decyl,N,N-dimethyl-, hexanedioate(2:1) | 2705260-06-3 | DDAC/Bardac22 (=DDAC) but no BCF reported |

* add the dossier-number in this link at X [https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/X](https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/X) to retrieve the information submitted on Bioaccumulation in the section on Environmental fate & pathways. Text in red indicates that the dossier refers to an in vivo BCF study.
Table S2: Chemicals

| Abbr. | Name                                      | CAS #     | Supplier     | Purity (%) | Internal std. for quantifying |
|-------|-------------------------------------------|-----------|--------------|------------|------------------------------|
| P9    | Nonylamine                                | 112-20-9  | TCI          | >99.5      | Q10IS                        |
| P12   | Dodecylamine                              | 929-73-7  | Acros        | >98.5      | Q10IS                        |
| P13   | Tridecylamine                             | 2869-34-3 | TCI          | >98        | Q14IS                        |
| P16   | Hexadecylamine                            | 143-27-1  | Sigma-Aldrich| >98.5      | P16IS                        |
| S12   | N-methyldodecylamine                      | 7311-30-0 | Sigma-Aldrich| 97         | Q10IS                        |
| S16   | N-methylhexadecylamine                    | 13417-08-8| Sigma-Aldrich| 95         | P16IS                        |
| T9    | N,N-dimethylnonylamine                    | 17373-27-2| Sigma-Aldrich| 97         | Q10IS                        |
| T10   | N,N-dimethyldecylamine                    | 1120-24-7 | TCI          | >93        | Q10IS                        |
| T13   | N,N-dimethyltridecylamine                 | 17373-29-4| Sigma-Aldrich| >97        | Q14IS                        |
| T14   | N,N-dimethyltetradecylamine               | 112-75-4  | Sigma-Aldrich| >95        | Q14IS                        |
| Q10   | N,N,N-trimethyl-1-decylammonium          | 2082-84-0 | Sigma-Aldrich| >98        | Q10IS                        |
| Q14   | N,N,N-trimethyl-1-tetradecylammonium     | 4574-04-3 | Sigma-Aldrich| >98        | Q14IS                        |
| P16IS | D_{33}-Hexadecylamine                     | 1191245-82-5 | QMX-Laboratories | 98   |
| Q10IS | D_{21}-N,N,N-trimethyl-1-decylammonium   | 2082-84-0 | QMX-Laboratories | 98   |
| Q14IS | D_{29}-N,N,N-trimethyl-1-tetradecylammonium | 95523-73-2 | QMX-Laboratories | 98   |
Table S3: Test chemical properties

| Abbr. | pKa$^\$ | Log D$_{OC}$§ | Log D$_{ML,W,C}^#$ | SMILES |
|-------|---------|----------------|-------------------|--------|
| P9    | 10.6    | 2.95           | 3.72             | CCCCCCCCCCN |
| P12   | 10.6    | 3.63           | 5.58             | CCCCCCCCCCCCCCN |
| P13   | 10.6    | 3.81           | 6.16             | CCCCCCCCCCCCCCNC |
| P16   | 10.6    | 4.46           | 7.90             | CCCCCCCCCCCCCCCCN |
| S12   | 10.8    | 3.49           | 5.39             | CCCCCCCCCCCCCNC |
| S16   | 10.8    | 4.35           | 7.71             | CCCCCCCCCCCCCCCCNC |
| T9    | 10      | 3.06           | 3.07             | CCCCCCCCCCN(C)C |
| T10   | 10      | 2.96           | 3.65             | CCCCCCCCCCN(C)C |
| T13   | 10      | 3.60           | 5.88             | CCCCCCCCCCCCCCN(C)C |
| T14   | 10      | 3.82           | 6.46             | CCCCCCCCCCCCCCN(C)C |
| Q10   | 10      | 2.92           | 3.34             | CCCCCCCCCC[N+](C)(C)C |
| Q14   | 10      | 3.78           | 5.46             | CCCCCCCCCCCCCC[N+](C)(C)C |

$^\$ organic carbon-water distribution ratio, calculated according to Droge and Goss (2013) 
*Environ. Sci. Technol.* 47, 798-806.

#$^\$ membrane-water partition coefficient of the charged form, from Timmer and Droge (2017)  
*Environ. Sci. Technol.* 51, 2890–2898, extrapolated for P13, P16, S16, T13, T14.

$pKa$ values derived/reported for different alkylamine type analogues. The selected value is expected to be independent of chain length for all longer chain homologues, using a weighted value obtained from the different sources with more focus on experimental data:

| Algorithm / simulated data | Experimental reference data (25°C) |
|---------------------------|-----------------------------------|
| Chem/Axon/Chemic-alize.org | ACD/Labs/COSMO-therm/selected |
| 10.21/10.7/10.75/10.5     | 10.79/(P10)/10.64 hexylamine (Perrin 1965) |
| Alkylamines               | 10.0/10.79 octylamine (SRC) |
|                           | 11.2/P16/10.6 octylamine (REACH) |
|                           | (https://echa.europa.eu/registration-dossier/-/registered-dossier/1996/4/22) |
| N-methylalkylamines       | 10.54/10.78/10.9/10.78/(S12) |
|                           | 10.9 N-methylbutylamine (Perrin 1972) |
| N,N-dimethylalkylamines   | 9.79/9.79/9.63/(T14) |
|                           | 9.99 N,N-dimethylpropylamine |
|                           | 10.02 N,N-dimethylbutylamine |
|                           | (H. K. Hall 1957, Correlation of the Base Strengths of Amines 1, JACS, 79 (20) 5441-5444) doi: 10.1021/JA01577A030) |
**Table S4:** Solvents

| Solvent            | Purpose                                                                 | Grade     | Supplier   |
|--------------------|-------------------------------------------------------------------------|-----------|------------|
| Methanol           | Test chemical solution for aquaria, extraction, SPE column, UPLC mobile phase | Lichrosolve | Merck      |
| Milli-Q water      | SPE column, dilution of purified extract, UPLC mobile phase             |           |            |
| Ammonium hydroxide | Adjust pH of Milli-Q                                                     |           | ACS        |
| Trifluoroacetic acid| SPE column                                                             | HiPerSolve Chroma-norm for LC/MS | VWR Chemicals |
| Ammonium acetate   | UPLC mobile phase                                                      | p.a.      | Merck      |
Table S5: Sampling schedule

| Day | hours | Water | Fish | TOC | Alkalinity |
|-----|-------|-------|------|-----|------------|
| 1   | 0     | X     |      |     |            |
| 1   | 2     |       | X    |     |            |
| 1   | 4     |       | X    |     |            |
| 1   | 6     | X     | X    | X   |            |
| 1   | 8     |       | X    |     |            |
| 2   | 24    | X     | X    | X   | X          |
| 2   | 28    |       | X    |     |            |
| 2   | 36    | X     | X    |     |            |
| 3   | 48    | X     | X    | X   |            |
| 4   | 72    |       | X    |     |            |
| 5   | 96    |       | X    |     |            |
| 6   | 120   |       | X    |     |            |
| 7   | 144   | X     | X    | X   | X          |
| 8   | 168   | X     | X    |     |            |
| 9   | 192   |       | X    |     |            |
| 10  | 216   | X     | X    | X   |            |
| 11  | 240   |       | X    |     |            |
| 12  | 264   |       | X    |     |            |
| 13  | 288   | X     | X    | X   |            |
| 14  | 312   | X     | X    |     | X          |
| 15  | 336   | X     | X    |     |            |

Transfer to elimination aquarium

| 15  | 336.5 | X     |
| 15  | 338   | X     |
| 15# | 340   | X     |
| 15  | 342   | X     | X    |
| 15# | 344   | X     |
| 16  | 360   | X     | X    | X    |
| 17  | 384   | X     | X    |
| 18  | 408   | X     | X    |
| 21  | 480   | X     | X    | X    |
| 24  | 552   | X     |
| 28  | 648   | X     |
| 32  | 744   | X     | X    |
| 37  | 864   | X     |
| 43  | 1008  | X     | X    |

# MIX1pH only
**Table S6:** Mean relative standard deviation (in %) of test substance concentration in triplicate water samples collected during the exposure phase (n = 21 × 3)

| MIX1 | Mean RSD<sup>a</sup> | MIX1pH | Mean RSD<sup>b</sup> | MIX2 | Mean RSD<sup>c</sup> |
|------|-----------------------|--------|-----------------------|------|-----------------------|
| P9   | 2                     | P9     | 3                     | T9   | 3                     |
| T10  | 6                     | T10    | 5                     | Q10  | 1                     |
| P12  | 2                     | P12    | 3                     | S12  | 2                     |
| T13  | 2                     | T13    | 3                     | P13  | 3                     |
| Q14  | 2                     | Q14    | 2                     | T14  | 3                     |
| P16  | 4                     | P16    | 4                     | S16  | 5                     |

<sup>a</sup> Outliers: 1 sample for all analytes (sampling error), 1 sample for P12, T13, Q14, P16 (particle artifact)

<sup>b</sup> Outliers: 1 sample for P12, 3 samples for T13 and Q14, 4 samples for P16 (particle artifact)

<sup>c</sup> Outliers: 4 samples for S12, P13, T14, S16 (particle artifact)

**Table S7:** Test substance concentration range (µg L<sup>-1</sup>) in blank samples from the water analysis (n = 12 for each mixture)

| MIX1 | Blank range | MIX1pH | Blank range | MIX2 | Blank range |
|------|-------------|--------|-------------|------|-------------|
| P9   | 0.04-0.35   | P9     | 0.06-0.28   | T9   | 0.03-0.35   |
| T10  | nd<sup>a</sup> | T10    | nd-0.1      | Q10  | 0.03-0.33   |
| P12  | 0.04-0.57   | P12    | 0.03-0.25   | S12  | 0.02-0.05   |
| T13  | 0.02-0.15   | T13    | nd-0.03     | P13  | 0.02-0.08   |
| Q14  | 0.07-0.71   | Q14    | 0.04-0.16   | T14  | nq<sup>b</sup> |
| P16  | nd-0.17     | P16    | nd          | S16  | nq          |

<sup>a</sup> nd = non-detect

<sup>b</sup> nq = not quantified

**Table S8:** Mean relative difference (in %) of test substance concentration in duplicate analyses of raw fish extracts

| MIX1 (n = 16) | MIX2 (n = 3) |
|---------------|--------------|
| P9            | T9           | 3             |
| T10           | 26<sup>a</sup>| Q10           | 7             |
| P12           | 5            | S12           | 2             |
| T13           | 2            | P13           | 7             |
| Q14           | 2            | T14           | 2             |
| P16           | 7            | S16           | 3             |

<sup>a</sup> One of the duplicate samples was strongly impacted by blanks and the concentrations lay between the LOD and LOQ.
**Table S9:** Mean relative difference of test substance concentration in duplicate analyses of fish homogenate

| MIX1 (n = 3) | MIX2 (n = 0) |
|--------------|--------------|
| P9           | <LOD         |
| T10          | 16           |
| P12          | 6            |
| T13          | 1            |
| Q14          | 3            |
| P16          | 5            |

**Table S10:** Recovery (mean ± standard deviation in %) of test chemicals spiked to fish homogenate or raw extract

| MIX1 | Recovery (%) | MIX2 | Recovery (%) |
|------|--------------|------|--------------|
| P9   | 92±6         | T9   | 103±6        |
| T10  | 100±3        | Q10  | 95±3         |
| P12  | 98±2         | S12  | 106±4        |
| T13  | 97±4         | P13  | 99±4         |
| Q14  | 92±7         | T14  | 106±2        |
| P16  | 106±3        | S16  | 116±7        |

**Table S11:** Limit of quantification (LOQ, ng g⁻¹) for fish analysis (determined as 10 × standard deviation in procedural blanks and control fish)

| MIX1 | LOQ | MIX2 | LOQ |
|------|-----|------|-----|
| P9   | 3   | T9   | 4   |
| T10  | 3   | Q10  | 4   |
| P12  | 4   | S12  | 7   |
| T13  | 5   | P13  | 8   |
| Q14  | 4   | T14  | 6   |
| P16  | 7   | S16  | 9   |
Table S12: Alkalinity and pH during the MIX1$ and MIX2 experiments

| Day  | hour | pH   | Alkalinity (mmol L$^{-1}$) |
|------|------|------|----------------------------|
|      |      | MIX1 | MIX2 | MIX1 | MIX2 |
| Exposure |  |  |  |  |  |
| 1    | 6   | 7.58 | 7.57 | 1.3137 | 1.3128 |
| 2    | 24  | 7.57 | 7.59 | 1.2969 | 1.2905 |
| 7    | 144 | 7.64 | 7.59 | 1.2977 | 1.2990 |
| 14   | 312 | 7.66 | 7.72 | 1.2663 | 1.2689 |
| Elimination |  |  |  |  |  |
| 16   | 360 | 7.78 | 7.75 | 1.2680 | 1.2705 |
| 21   | 480 | 7.75 | 7.75 | 1.2987 | 1.3012 |
| 32   | 744 | 7.81 | 7.81 | 1.3017 | 1.2951 |
| 43   | 1008| 7.9  | 7.92 | 1.2177 | 1.2131 |

$\text{During the MIX1pH experiment alkalinity was strongly affected by the addition of formic acid.}$
Table S13: TOC during the MIX1\textsuperscript{a} and MIX2 experiments\textsuperscript{b}

| Day | hours | TOC (mg C L\textsuperscript{-1}) |
|-----|-------|-------------------------------|
|     |       | MIX1  | MIX2  |
| Exposure |       | MIX1  | MIX2  |
| 2   | 24    | 5.7   | 6.2   |
| 3   | 48    | 5.8   | 5.7   |
| 4   | 72    | 5.4   | 5.7   |
| 5   | 96    | 5.5   | 5.8   |
| 6   | 120   | 5.3   | 5.4   |
| 7   | 144   | 6.1   | 5.9   |
| 8   | 168   | 5.4   | 5.6   |
| 9   | 192   | 5.2   | 5.7   |
| 10  | 216   | 5.6   | 5.7   |
| 11  | 240   | 5.1   | 5.4   |
| 12  | 264   | 5.2   | 5.2   |
| 13  | 288   | 5.3   | 5.1   |
| 14  | 312   | 5.6   | 5.4   |
| Elimination |       | MIX1  | MIX2  |
| 15  | 342   | 5.1   | 4.7   |
| 16  | 360   | 5.1   | 5.2   |

\textsuperscript{a} During the MIX1pH experiment TOC was strongly affected by the addition of formic acid.

\textsuperscript{b} There was little variability during the experiment or between experiments. The methanol from the infusion solution contributed 0.8 mg C L\textsuperscript{-1} to the TOC, while the remainder originated from the fish, the fish feed and organic carbon in the raw water supply (~4 mg C L\textsuperscript{-1}), some of which passed through the activated carbon filters in the aquarium water treatment system. In the MIX1pH experiment meaningful measurements of TOC were not possible due to the background signal from the formic acid.
**Table S14**: Concentrations (µg L\(^{-1}\)) of test chemicals in water during the MIX1 experiment. Each data point is the mean of triplicate samples.

| Day | Exposure | P9  | T10 | P12 | T13 | Q14 | P16 |
|-----|----------|-----|-----|-----|-----|-----|-----|
| 1   | 0        | 118.8 | 21.6 | 29.0 | 7.4 | 22.8 | 1.41 |
| 1   | 2        | 111.1 | 20.1 | 25.0 | 6.7 | 23.2 | 1.37 |
| 1   | 4        | 119.3 | 19.6 | 24.8 | 6.5 | 25.3 | 1.45 |
| 1   | 6        | 110.7 | 18.9 | 23.8 | 6.4 | 24.6 | 1.41 |
| 1   | 8        | 111.9 | 19.6 | 23.2 | 6.5 | 25.1 | 1.43 |
| 2   | 24       | 109.1 | 18.9 | 24.3 | 7.0 | 26.2 | 1.50 |
| 2   | 28       | 115.4 | 19.6 | 24.6 | 7.1 | 26.5 | 1.47 |
| 2   | 36       | 109.1 | 19.3 | 24.2 | 7.0 | 26.6 | 1.49 |
| 3   | 48       | 110.1 | 19.8 | 24.4 | 7.2 | 25.3 | 1.46 |
| 4   | 72       | 107.9 | 19.2 | 24.4 | 7.2 | 25.1 | 1.41 |
| 5   | 96       | 108.4 | 19.0 | 24.4 | 7.4 | 25.8 | 1.49 |
| 6   | 120      | 108.0 | 19.3 | 24.8 | 7.8 | 25.7 | 1.49 |
| 7   | 144      | 105.5 | 19.4 | 24.0 | 7.3 | 25.2 | 1.41 |
| 8   | 168      | 104.8 | 19.3 | 23.8 | 7.7 | 25.6 | 1.52 |
| 9   | 192      | 108.7 | 18.5 | 24.6 | 7.7 | 26.3 | 1.51 |
| 10  | 216      | 102.5 | 18.8 | 23.8 | 7.7 | 25.3 | 1.61 |
| 11  | 240      | 102.8 | 18.0 | 24.2 | 7.8 | 25.4 | 1.63 |
| 12  | 264      | 98.2  | 19.5 | 22.9 | 7.8 | 24.3 | 1.58 |
| 13  | 288      | 96.7  | 17.3 | 21.9 | 7.0 | 23.7 | 1.55 |
| 14  | 312      | 94.9  | 19.4 | 22.8 | 7.5 | 24.5 | 1.73 |
| 15  | 336      | 97.5  | 17.4 | 22.2 | 7.3 | 23.9 | 1.54 |

|     | Mean     | 107 | 19.2 | 24.2 | 7.2 | 25.1 | 1.50 |
|-----|----------|-----|-----|-----|-----|-----|-----|
|     | RSD (%)  | 6.3 | 4.7 | 5.8 | 6.2 | 4.1 | 5.8 |
|     | Target   | 100 | 25  | 25  | 10  | 25  | 2.5 |
|     | % of target | 107 | 77  | 97  | 72  | 100 | 60  |

| Elimination |
|-------------|
| 15  | 336.5 | 0.57 | 0.54 | 0.82 | 0.20 | 0.35 | 0.10 |
| 15  | 338  | 0.70 | 0.83 | 0.99 | 0.30 | 0.34 | 0.11 |
| 15  | 342  | 0.46 | 0.81 | 0.79 | 0.28 | 0.27 | 0.08 |
| 16  | 360  | 0.12 | 0.13 | 0.40 | 0.16 | 0.23 | 0.06 |
| 17  | 384  | 0.21 | 0.08 | 0.36 | 0.14 | 0.25 | 0.08a |
| 18  | 408  | 0.13 | 0.36 | 0.14 | 0.32 | 0.07 |
| 21  | 480  | 0.09 | 0.19 | 0.06 | 0.15 | 0.06 |
| 32  | 744  | 0.02 | 0.11 | 0.03 | 0.12 | 0.06 |
| 43  | 1008 | 0.07 | 0.05 | 0.01 | 0.08 |

a Concentrations blank corrected, lie between LOD and LOQ
Table S15: Concentrations (µg L⁻¹) of test chemicals in water during the MIX2 experiment. Each data point is the mean of triplicate samples.

| Day | hours | T9  | Q10 | S12 | P13 | T14 | S16 |
|-----|-------|-----|-----|-----|-----|-----|-----|
| 1   | 0     | 52  | 56  | 23.8| 6.4 | 1.37| 1.22|
| 1   | 2     | 49  | 55  | 21.0| 6.2 | 1.61| 1.46|
| 1   | 4     | 49  | 56  | 20.1| 5.7 | 1.45| 1.36|
| 1   | 6     | 50  | 56  | 20.1| 6.0 | 1.56| 1.52|
| 1   | 8     | 51  | 57  | 20.8| 6.3 | 1.69| 1.67|
| 2   | 24    | 49  | 56  | 22.1| 6.6 | 1.68| 1.51|
| 2   | 28    | 54  | 58  | 21.9| 6.4 | 1.70| 1.51|
| 2   | 36    | 53  | 57  | 22.4| 6.3 | 1.60| 1.43|
| 3   | 48    | 51  | 55  | 22.7| 6.7 | 1.80| 1.53|
| 4   | 72    | 49  | 54  | 22.2| 6.5 | 1.66| 1.50|
| 5   | 96    | 50  | 55  | 22.9| 6.7 | 1.79| 1.64|
| 6   | 120   | 51  | 55  | 22.7| 6.4 | 1.56| 1.66|
| 7   | 144   | 50  | 54  | 21.9| 6.4 | 1.67| 1.72|
| 8   | 168   | 52  | 55  | 22.4| 6.5 | 1.80| 1.65|
| 9   | 192   | 51  | 57  | 23.1| 6.6 | 1.82| 1.61|
| 10  | 216   | 50  | 54  | 22.2| 6.3 | 1.80| 1.60|
| 11  | 240   | 50  | 55  | 23.4| 6.7 | 1.83| 1.74|
| 12  | 264   | 49  | 53  | 22.0| 6.3 | 1.74| 1.69|
| 13  | 288   | 47  | 52  | 21.3| 6.0 | 1.59| 1.79|
| 14  | 312   | 48  | 54  | 21.9| 6.5 | 1.65| 1.89|
| 15  | 336   | 51  | 55  | 22.7| 6.9 | 1.84| 1.85|

|            | Mean  | RSD (%) | Target | % of target |
|------------|-------|---------|--------|-------------|
|            | 50    | 3.1     | 50     | 100         |
|            | 55    | 2.3     | 50     | 110         |
|            | 22.1  | 4.4     | 25     | 88          |
|            | 6.4   | 4.1     | 10     | 64          |
|            | 1.68  | 7.5     | 2.5    | 68          |
|            | 1.60  | 10.2    | 2.5    | 64          |

|            | Elimination |
|------------|-------------|
| 15         | 338         | 0.89      | 0.03    | 0.96      | 0.22      | 0.05      | 0.08     |
| 15         | 342         | 0.76      | 0.05    | 1.03      | 0.16      | 0.04      | 0.05     |
| 16         | 360         | 0.06      | 0.05    | 0.53      | 0.11      | 0.03      | 0.04     |
| 17         | 384         | 0.03      | 0.08    | 0.36      | 0.09      | 0.03      | 0.04     |
| 18         | 408         | 0.03      | 0.04    | 0.28      | 0.09      | 0.03      | 0.04     |
| 21         | 480         | 0.02      | 0.05    | 0.11      | 0.06      | 0.02      | 0.03     |
| 32         | 744         | 0.03      | 0.05    | 0.04      | 0.05      | 0.02      | 0.04     |
| 43         | 1008        | 0.02      | 0.02    | 0.04      |           |           |          |
Table S16: Concentrations (µg L\(^{-1}\)) of test chemicals in water during the MIX1pH experiment. Each data point is the mean of triplicate samples.

| Day | hours | P9  | T10 | P12 | T13 | Q14 | P16 |
|-----|-------|-----|-----|-----|-----|-----|-----|
|     |       |     |     |     |     |     |     |
| Exposure | | | | | | | |
| 1   | 0     | 119 | 23.6| 30.4| 7.1 | 22.9| 1.37|
| 1   | 2     | 116 | 22.0| 28.4| 6.9 | 23.9| 1.38|
| 1   | 4     | 108 | 20.8| 26.8| 6.9 | 24.4| 1.38|
| 1   | 6     | 121 | 24.7| 31.5| 8.3 | 28.2| 1.63|
| 1   | 8     | 115 | 23.9| 30.1| 8.0 | 26.5| 1.66|
| 2   | 24    | 112 | 22.8| 29.6| 7.9 | 26.6| 1.54|
| 2   | 36    | 118 | 24.2| 30.9| 8.1 | 25.8| 1.56|
| 3   | 48    | 116 | 23.0| 30.3| 8.3 | 27.3| 1.68|
| 4   | 72    | 108 | 22.1| 28.5| 8.2 | 27.2| 1.59|
| 5   | 96    | 110 | 22.2| 29.4| 8.0 | 26.4| 1.71|
| 6   | 120   | 107 | 22.0| 28.2| 7.8 | 26.1| 1.58|
| 7   | 144   | 107 | 21.2| 27.7| 7.9 | 26.1| 1.64|
| 8   | 168   | 112 | 22.5| 29.6| 8.4 | 27.5| 1.68|
| 9   | 192   | 114 | 23.3| 29.1| 8.2 | 26.5| 1.70|
| 10  | 216   | 111 | 21.4| 27.9| 7.7 | 25.0| 1.46|
| 11  | 240   | 109 | 21.2| 28.6| 7.9 | 26.0| 1.51|
| 12  | 264   | 115 | 22.1| 29.1| 7.8 | 26.2| 1.60|
| 13  | 288   | 108 | 21.0| 26.3| 7.2 | 24.1| 1.37|
| 14  | 312   | 115 | 21.9| 28.5| 8.0 | 25.6| 1.44|
| 15  | 336   | 112 | 21.9| 28.8| 8.1 | 25.3| 1.43|
| Mean|       | 113 | 22.4| 29.0| 7.8 | 25.9| 1.55|
| RSD (%)|   | 3.7 | 4.9 | 4.5 | 5.8 | 5.1 | 7.7 |
| Target|     | 100 | 25  | 25  | 10  | 25  | 2.5 |
| % of target| | 113 | 90  | 116 | 78  | 104 | 62 |

*Elimination*\(^a\)

| Day | hours | P9  | T10 | P12 | T13 | Q14 | P16 |
|-----|-------|-----|-----|-----|-----|-----|-----|
| 15  | 336.5 | 0.28| 0.37| 0.32| 0.16| 0.31| 0.05|
| 15  | 338   | 0.25| 0.51| 0.54| 0.34| 0.28| 0.09|
| 15  | 340   | 0.28| 0.54| 0.59| 0.38| 0.33| 0.10|
| 15  | 342   | 0.17| 0.49| 0.46| 0.34| 0.21| 0.13|
| 15  | 344   | 0.20| 0.52| 0.50| 0.33| 0.33| 0.13|
| 16  | 360   | 0.17| 0.27| 0.23| 0.14| 0.21| 0.08|
| 17  | 384   | 0.17| 0.19| 0.17| 0.08| 0.20| 0.06\(^b\)|
| 18  | 408   | 0.07| 0.16| 0.07| 0.05| 0.12| 0.05|
| 21  | 480   | 0.07| 0.12| 0.06| 0.03| 0.10| 0.04|
| 28  | 648   | 0.07| 0.07| 0.06| 0.02| 0.18| 0.03|
| 32  | 744   | 0.13| 0.08| 0.07| 0.03| 0.12| 0.07|
| 43  | 1008  | 0.09| 0.06| 0.01| 0.01| 0.09| 0.09|
During the MIX1pH experiment a higher sampling frequency was employed at the beginning of the elimination phase, and a pulse in the concentration of T10, P12, and T13 in water was observed during the first hours after adding the fish to the elimination aquarium. This was presumably caused by depuration of the test chemicals by the fish. However, within 2 days the concentrations of all chemicals had fallen to a level at least a factor of 50 below the concentrations in the exposure phase. The one exception was P16, for which the decrease was a factor of ~20.

Concentrations blank corrected, lie between LOD and LOQ
Table S17: Concentrations (ng g⁻¹ ww) of test chemicals in fish during the exposure phase of the MIX1 experiment.

| Day | hours | P9  | T10  | P12  | T13  | Q14 | P16 |
|-----|-------|-----|------|------|------|-----|-----|
| 1   | 6     | 46  | 1005 | 156  | 1276 | 57  | 67  |
| 1   | 6     | 369 | 1035 | 2949 | 1400 | 49  | 270 |
| 1   | 6     | 195 | 1204 | 2736 | 1684 | 47  | 292 |
| 2   | 24    | 254 | 2026 | 5200 | 3828 | 75  | 793 |
| 2   | 24    | 55  | 1827 | 751  | 4003 | 92  | 432 |
| 2   | 24    | 349 | 1983 | 6604 | 4074 | 89  | 968 |
| 2   | 36    | 355 | 1748 | 7711 | 4936 | 95  | 1371|
| 2   | 36    | 292 | 2351 | 7715 | 5990 | 123 | 1412|
| 3   | 48    | 57  | 1745 | 763  | 6741 | 149 | 843 |
| 3   | 48    | 150 | 2149 | 5457 | 5935 | 124 | 1538|
| 3   | 48    | 77  | 2102 | 1166 | 7114 | 174 | 799 |
| 7   | 144   | 487 | 2041 | 10816| 11202| 319 | 3525|
| 7   | 144   | 235 | 2492 | 5563 | 12950| 334 | 2947|
| 7   | 144   | 165 | 2535 | 3782 | 13222| 309 | 3101|
| 10  | 216   | 693 | 2751 | 14523| 14667| 455 | 4999|
| 10  | 216   | 856 | 3473 | 19268| 17276| 580 | 6184|
| 10  | 216   | 693 | 3211 | 14716| 14121| 501 | 4557|
| 13  | 288   | 547 | 2826 | 13992| 18432| 689 | 5951|
| 13  | 288   | 154 | 2919 | 3812 | 16588| 635 | 3296|
| 13  | 288   | 976 | 3529 | 19113| 20280| 554 | 7050|
| 15  | 336   | 115 | 2762 | 1598 | 19788| 734 | 2026|
| 15  | 336   | 129 | 2951 | 2448 | 21365| 563 | 3217|
| 15  | 336   | 405 | 3258 | 9703 | 16864| 581 | 5441|
Table S18: Concentrations (ng g\(^{-1}\) ww) of test chemicals in fish during the elimination phase of the MIX1 experiment.

| Day | hours | P9  | T10 | P12 | T13 | Q14 | P16 |
|-----|-------|-----|-----|-----|-----|-----|-----|
| 15  | 342   | 299 | 1359| 16257| 15495| 766 | 5769|
| 15  | 342   | 25  | 1608| 1371 | 20065| 547 | 3474|
| 15  | 342   | 11.7| 1227| 2571 | 13970| 610 | 4530|
| 16  | 360   | 8.4 | 586 | 2022 | 19163| 528 | 5433|
| 16  | 360   | 59  | 445 | 13516| 17776| 515 | 7372|
| 16  | 360   | 5.1 | 256 | 3296 | 14430| 639 | 5047|
| 17  | 384   | 121 | 988 | 13423| 525  | 3936|
| 17  | 384   | 88  | 1944| 12902| 576  | 4225|
| 17  | 384   | 92  | 10328| 13740| 510  | 5785|
| 18  | 408   | 36  | 1314| 11513| 621  | 4346|
| 18  | 408   | 83  | 4967| 16628| 590  | 5435|
| 18  | 408   | 53  | 3015| 12977| 588  | 3698|
| 21  | 480   | 12  | 1614| 8334 | 446  | 3112|
| 21  | 480   | 12  | 451 | 6680 | 564  | 3040|
| 21  | 480   | 12  | 295 | 10632| 445  | 2566|
| 24  | 552   | 10  | 175 | 6960 | 471  | 1943|
| 24  | 552   | 5   | 688 | 7893 | 347  | 3572|
| 24  | 552   | 10  | 3968| 4552 | 385  | 4369|
| 28  | 648   |     | 1648| 4095 | 326  | 3692|
| 28  | 648   |     | 261 | 3077 | 309  | 2667|
| 28  | 648   |     | 2010| 5341 | 389  | 2867|
| 32  | 744   |     | 123 | 2211 | 231  | 1831|
| 32  | 744   |     | 132 | 2563 | 281  | 1801|
| 32  | 744   |     | 261 | 1581 | 249  | 1547|
| 37  | 864   |     | 66  | 1789 | 290  | 1140|
| 37  | 864   |     | 70  | 1887 | 275  | 1755|
| 43  | 1008  |     | 321 | 802  | 160  | 1897|
| 43  | 1008  |     | 20  | 882  | 199  | 1449|
| 43  | 1008  |     | 141 | 1171 | 264  | 1429|
Table S19: Concentrations (ng g\(^{-1}\) ww) of test chemicals in fish during the exposure phase of the MIX2 experiment.

| Day | hours | T9   | Q10 | S12 | P13 | T14 | S16 |
|-----|-------|------|-----|-----|-----|-----|-----|
| 1   | 6     | 714  | 5.9 | 2044| 155 | 316 | 224 |
| 1   | 6     | 801  | 0.6 | 2504| 430 | 223 | 234 |
| 1   | 6     | 813  | 1.8 | 1851| 80  | 342 | 232 |
| 2   | 24    | 1101 | 2.1 | 4312| 439 | 947 | 814 |
| 2   | 24    | 1289 | 4.6 | 5804| 856 | 1207| 946 |
| 2   | 24    | 1143 | 6.1 | 5488| 744 | 972 | 1072|
| 2   | 36    | 1400 | 4.1 | 6907| 1054| 1508| 1383|
| 2   | 36    | 1296 | 3.6 | 5723| 484 | 1452| 1250|
| 3   | 48    | 1749 | 6.1 | 8412| 2242| 2149| 2352|
| 3   | 48    | 1108 | 4.5 | 5529| 722 | 1951| 1723|
| 3   | 48    | 1411 | 3.7 | 7647| 968 | 2007| 1683|
| 7   | 144   | 1576 | 5.4 | 11933| 2695| 5383| 4415|
| 7   | 144   | 1408 | 10.8| 11110| 3511| 4807| 4334|
| 7   | 144   | 1526 | 6.7 | 11889| 2666| 5108| 5117|
| 10  | 216   | 2284 | 11.9| 17361| 13542| 8085| 6821|
| 10  | 216   | 1616 | 7.6 | 14240| 11565| 6461| 5672|
| 10  | 216   | 2214 | 13.2| 16321| 4310 | 7885| 6241|
| 13  | 288   | 2180 | 10.9| 18782| 4229 | 9510| 13655|
| 13  | 288   | 2281 | 11.9| 16935| 4176 | 8002 | 7286|
| 13  | 288   | 3026 | 12.4| 20005| 14531| 8874| 7356|
| 15  | 336   | 2042 | 8.7 | 16795| 2672 | 10008| 7785|
| 15  | 336   | 1933 | 11.4| 15143| 1861 | 7934 | 7156|
| 15  | 336   | 2292 | 13  | 17482| 6121 | 8980 | 9853|

# Concentrations blank corrected, lie between LOD and LOQ
Table S20: Concentrations (ng g\(^{-1}\) ww) of test chemicals in fish during the elimination phase of the MIX2 experiment.

| Day | hours | T9  | Q10 | S12 | P13 | T14 | S16 |
|-----|-------|-----|-----|-----|-----|-----|-----|
| 15  | 342   | 741 | 12  | 16392 | 6685 | 10305 | 8035 |
| 15  | 342   | 920 | 8   | 12294 | 2829 | 9311  | 7043 |
| 15  | 342   | 847 | 7   | 20378 | 15761 | 10236 | 8107 |
| 16  | 360   | 519 | 9   | 10773 | 6949 | 6790  | 6367 |
| 16  | 360   | 174 | 3.9 | 12920 | 5087 | 7392  | 9085 |
| 16  | 360   | 660 | 12  | 11666 | 5334 | 7294  | 6500 |
| 17  | 384   | 73  |     | 9592  | 10407 | 8218  | 6091 |
| 17  | 384   | 133 |     | 9481  | 4936  | 6771  | 6012 |
| 17  | 384   | 52  |     | 9797  | 10347 | 6472  | 5634 |
| 18  | 408   | 17.5|     | 10492 | 12394 | 8870  | 6030 |
| 18  | 408   | 33.4|     | 8083  | 3833  | 7510  | 5748 |
| 18  | 408   | 46.8|     | 9560  | 10057 | 7958  | 5763 |
| 21  | 480   |     |     | 4065  | 1198  | 5735  | 4548 |
| 21  | 480   |     |     | 4742  | 5609  | 5121  | 4560 |
| 21  | 480   |     |     | 4901  | 2001  | 6208  | 5414 |
| 24  | 552   |     |     | 2728  | 3550  | 4533  | 4489 |
| 24  | 552   |     |     | 3468  | 2144  | 4126  | 5430 |
| 24  | 552   |     |     | 2401  | 1319  | 5100  | 4676 |
| 28  | 648   |     |     | 1471  | 2224  | 3780  | 3059 |
| 28  | 648   |     |     | 1951  | 2850  | 3329  | 3171 |
| 28  | 648   |     |     | 2022  | 1249  | 3164  | 3912 |
| 32  | 744   |     |     | 1808  | 2377  | 4233  | 3350 |
| 32  | 744   |     |     | 1434  | 2481  | 2656  | 2933 |
| 32  | 744   |     |     | 1287  | 1884  | 1711  | 2522 |
| 37  | 864   |     |     | 765   | 421   | 2057  | 2261 |
| 37  | 864   |     |     | 986   | 1350  | 1639  | 2441 |
| 37  | 864   |     |     | 616   | 1274  | 2017  | 2829 |
| 43  | 1008  |     |     | 675   | 1138  | 1680  | 2163 |
| 43  | 1008  |     |     | 164   | 136   | 783   | 1547 |
| 43  | 1008  |     |     | 215   | 709   | 955   | 1738 |

# Concentrations blank corrected, lie between LOD and LOQ
Table S21: Concentrations (ng g\(^{-1}\) ww) of test chemicals in fish during the exposure phase of the MIX1pH experiment.

| Day | hours | P9\(^{§}\) | T10  | P12  | T13  | Q14 | P16  |
|-----|-------|------------|------|------|------|-----|------|
| 1   | 6     | 32.0       | 69   | 106  | 445  | 58  | 169  |
| 1   | 6     | 34.7       | 80   | 399  | 435  | 45  | 260  |
| 2   | 24    | 34.5       | 149  | 108  | 1048 | 99  | 394  |
| 2   | 24    | 31.2       | 116  | 92   | 848  | 141 | 377  |
| 2   | 24    | 32.6       | 139  | 103  | 1058 | 135 | 362  |
| 2   | 36    | 31.4       | 146  | 149  | 1196 | 192 | 593  |
| 2   | 36    | 31.5       | 168  | 151  | 1271 | 156 | 510  |
| 3   | 48    | 23.7       | 185  | 1158 | 1795 | 181 | 1688 |
| 3   | 48    | 4.0        | 132  | 337  | 1512 | 204 | 1242 |
| 3   | 48    | 31.1       | 135  | 168  | 1390 | 248 | 904  |
| 7   | 144   | 34.4       | 203  | 205  | 2199 | 398 | 1769 |
| 7   | 144   | 38.9       | 177  | 133  | 2235 | 416 | 1245 |
| 7   | 144   | 37.9       | 236  | 156  | 2488 | 358 | 1586 |
| 10  | 216   | 34.8       | 267  | 142  | 2702 | 689 | 1823 |
| 10  | 216   | 30.0       | 176  | 176  | 2846 | 360 | 1755 |
| 10  | 216   | 31.5       | 156  | 170  | 2274 | 500 | 1531 |
| 13  | 288   | 49         | 238  | 1904 | 3100 | 475 | 5611 |
| 13  | 288   | 52         | 259  | 1829 | 3218 | 541 | 5426 |
| 13  | 288   | 46         | 209  | 181  | 2779 | 739 | 1873 |
| 15  | 336   | 44         | 208  | 206  | 2828 | 813 | 2374 |
| 15  | 336   | 31         | 62   | 187  | 2632 | 846 | 2061 |
| 15  | 336   | 44.2       | 186  | 475  | 2557 | 571 | 3284 |

§ Concentrations not blank corrected, blank 34±5 ng g\(^{-1}\) ww  
# Concentrations blank corrected, lie between LOD and LOQ  
\(^{a}\) Treated as an outlier in the model fitting
Table S22: Concentrations (ng g⁻¹ ww) of test chemicals in fish during the elimination phase of the MIX1 pH experiment.

| Day | hours | P9  | T10 | P12 | T13 | Q14 | P16 |
|-----|-------|-----|-----|-----|-----|-----|-----|
| 15  | 338   | 128 | 168 | 2567| 852 | 2142|     |
| 15  | 338   | 126 | 275 | 2821| 719 | 2968|     |
| 15  | 338   | 128 | 95  | 3012| 1367| 2240|     |
| 15  | 340   | 59  | 180 | 1767| 801 | 2183|     |
| 15  | 340   | 143 | 380 | 3251| 817 | 4320|     |
| 15  | 340   | 71  | 298 | 2549| 466 | 3627|     |
| 15  | 344   | 83  | 279 | 2525| 703 | 3690|     |
| 15  | 344   | 49  | 206 | 2010| 744 | 2564|     |
| 15  | 344   | 75  | 109 | 2446| 644 | 2564|     |
| 16  | 360   | 14.7| 26.6| 1728| 595 | 1287|     |
| 16  | 360   | 11.9| 46  | 1555| 794 | 2102|     |
| 17  | 384   | 6.3 | 31.2| 685 | 635 | 2094|     |
| 17  | 384   | 8.7 | 28.1| 1261| 594 | 1270|     |
| 17  | 384   | 8.7 | 9.1 | 837 | 731 | 2436|     |
| 18  | 408   | 4.9 | 11.4| 401 | 677 | 1453|     |
| 18  | 408   | 5.8 | 70  | 584 | 760 | 3667|     |
| 18  | 408   | 6.3 | 48  | 578 | 490 | 4044|     |
| 21  | 480   | 16.9| 311 | 382 | 2194|     |     |
| 21  | 480   | 4.5 | 6.8 | 406 | 532 | 2544|     |
| 21  | 480   | 4.0 | 18.7| 290 | 416 | 3621|     |
| 24  | 552   | 181 | 379 | 1331|     |     |     |
| 24  | 552   | 153 | 413 | 1634|     |     |     |
| 24  | 552   | 133 | 449 | 3011|     |     |     |
| 28  | 648   | 42  | 449 | 863 |     |     |     |
| 28  | 648   | 50  | 508 | 1802|     |     |     |
| 28  | 648   | 70  | 384 | 1677|     |     |     |
| 32  | 744   | 30.3| 309 | 1389|     |     |     |
| 32  | 744   | 37.7| 371 | 1464|     |     |     |
| 32  | 744   | 47  | 500 | 1495|     |     |     |
| 37  | 864   | 13.1| 203 | 621 |     |     |     |
| 37  | 864   | 30.9| 306 | 926 |     |     |     |
| 37  | 864   | 42  | 315 | 300 |     |     |     |
| 38  | 888   | 289 | 270 | 879 |     |     |     |
| 38  | 888   | 18.8| 224 | 459 |     |     |     |
| 38  | 888   | 20.0| 193 | 464 |     |     |     |
**Table S23**: Inter-individual variability of test chemical concentrations in fish, expressed as the relative standard deviation in % for the 3 fish sampled at each time point (mean across all time points ± standard deviation).

| MIX1   | RSD   | MIX1pH | RSD   | MIX2  | RSD   |
|--------|-------|--------|-------|-------|-------|
| P9     | 54±27 | P9     | T9    | 12±7  |
| T10    | 11±4  | T10    | 20±14 | Q10   | 34±30 |
| P12    | 61±37 | P12    | 47±40 | S12   | 12±5  |
| T13    | 10±3  | T13    | 9±3   | P13   | 55±22 |
| Q14    | 12±4  | Q14    | 18±7  | T14   | 10±6  |
| P16    | 31±20 | P16    | 23±16 | S16   | 15±11 |

**Table S24**: Course approximation of relative concentrations (with respect to the parent substance) of demethylation products of test chemicals found in 3 fish sampled at the end of the exposure period, assuming that response factors of parent and product were similar. The relative concentration is given in brackets below the identity of the product.

| Parent | T9 (1) | T10 (1) | S12 (1) | T13 (1) | T14 (1) | S16 (1) |
|--------|--------|--------|--------|--------|--------|--------|
| Product 1 | S9 (0.2) | S10 (0.15) | P12 (0.005) | S13 (0.05) | S14 (0.1) | P16 (nd) |
| Product 2 | P9 (0.02) | P10 (nd) | P13 (0.001) | P14 (nd) |        |        |
Table S25: Comparison of $k_U$ and $k_U/D_{MLW}$ for Q14, S16 and P16

| Chemical | Uptake rate constant ($k_U$, L kg$^{-1}$ h$^{-1}$) | $k_U/D_{MLW}$ |
|----------|---------------------------------|---------------|
|          | pH 7.6  | pH 6.2  | pH 7.6  | pH 6.2  |
| Q14      | 0.104   | 0.121   | 3.6E-07 | 4.2E-07 |
| S16      | 22      | 4.3E-07 |         |         |
| P16      | 15.3    | 7.8     | 1.9E-07 | 9.8E-08 |
Figure S1: Relative quantity extracted from fish homogenate in successive batch extractions at different temperatures. 500 mg of homogenate was extracted in 8 mL of methanol after addition of internal standard. The slurry was sonicated for 1 h followed by centrifugation, decanting of the extract, and re-extraction of the residue in fresh solvent and internal standard. The first extraction was conducted at 25 °C for 0.5 h followed by 50 °C for 0.5 h.

Figure S2: pH during the MIX1pH experiment. There positive spike during the elimination phase was due to a defect in the formic acid supply system. The period of elevated pH was ~6 h.
Figure S3: Semilogarithmic plot of chemical elimination during the MIX1 experiment.
Figure S4: Semilogarithmic plot of chemical elimination during the MIX2 experiment.
Figure S5: Semilogarithmic plot of chemical elimination during the MIX1pH experiment.
Figure S6: Plot of chemical uptake and elimination during the MIX1 (pH 7.6) experiment showing the model fit to the data.
Figure S7: Plot of chemical uptake and elimination during the MIX2 (pH 7.6) experiment showing the model fit to the data.
Figure S8: Plot of chemical uptake and elimination during the MIX1pH (pH 6.2) experiment showing the model fit to the data. All data for P9 were below the LOQ.