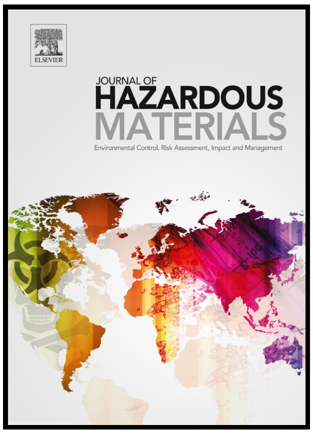
**Ebert, A.**, Fenner, K., Mayer, P., McLachlan, M.S., Scheringer, M., Tolls, J., Wania, F.,
**Goss, K.U.** (2026):
Comment on “By integrating previously overlooked drivers AI boosts bioaccumulation
assessment in fish”
*J. Hazard. Mater.* **415** , art. 142668

https://doi.org/10.1016/j.jhazmat.2026.142668



# Comment on “By integrating previously overlooked drivers AI boosts bioaccumulation assessment in fish”

Andrea Ebert
Kathrin Fenner
Philipp Mayer
Michael S. McLachlan
Martin Scheringer
Johannes Tolls
Frank Wania
Kai-Uwe Goss

PII: S0304-3894(26)01646-8
DOI: https://doi.org/10.1016/j.jhazmat.2026.142668
Reference: HAZMAT142668

To appear in: *Journal of Hazardous Materials*
Accepted date: 8

Please cite this article as: Andrea Ebert, Kathrin Fenner, Philipp Mayer, Michael S. McLachlan, Martin Scheringer, Johannes Tolls, Frank Wania and Kai-Uwe Goss, Comment on “By integrating previously overlooked drivers AI boosts bioaccumulation assessment in fish”, *Journal of Hazardous Materials,* (2026) doi:https://doi.org/10.1016/j.jhazmat.2026.142668

**Title:**

# Comment on “By integrating previously overlooked drivers AI boosts bioaccumulation assessment in fish”

**Authors and Affiliations**

Andrea Ebert[a,*], Kathrin Fenner[b,c], Philipp Mayer[d], Michael S. McLachlan[e] , Martin Scheringer[f], Johannes Tolls[g], Frank Wania[h], Kai-Uwe Goss [a,i]

---

[a] Department of Computational Biology & Chemistry, Helmholtz Centre for Environmental Research (UFZ), 04318 Leipzig, Germany
[b] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf 8600, Switzerland
[c] Department of Chemistry, University of Zürich, Zürich 8057, Switzerland
[d] Technical University of Denmark, Department of Environmental and Resource Engineering, Bygningstorvet, Building 115, 2800 Kongens Lyngby, Denmark
[e] Department of Environmental Science (ACES), Stockholm University, 10691 Stockholm, Sweden
[f] Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, 8092 Zürich, Switzerland
[g] Henkel AG & Co KGaA, Henkelstr. 67, 40589 Düsseldorf, Germany
[h] Department of Physical and Environmental Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, Ontario, Canada M1C 1A4
[i] Institute of Chemistry, University of Halle-Wittenberg, 06120 Halle, Germany

* corresponding author

Dear Editor,

In their recent contribution, Köhler et al. (2026) seek to address challenges associated with the sound determination, application and assessment of bioconcentration factors (BCFs). Whereas progress related to experimental, analytical, conceptual and modelling aspects of bioconcentration is welcome, we believe that the paper contains several severe thermodynamic misconceptions and technical flaws. These misconceptions lead the authors to draw conclusions that question established regulation of organic chemicals. While we do not object to regulations being critically evaluated, this must be done on a scientifically correct foundation.

In the following, we offer comments on some of the most important statements of the paper, with corresponding citations listed in the Supplementary Information (SI):

**Statement:** Octanol-water partition experiments are conducted at equal volumes of octanol and water, and only in this setting can correct equilibrium partitioning be expected.

**Comment:** This statement violates fundamental thermodynamic concepts, according to which partition constants are independent of phase volumes. It becomes immediately obvious from a series of partitioning experiments with different volumes that the partition coefficient is always the same. In practice, a specific volume ratio is chosen for experimental convenience and to ensure reliable analytical quantification, but this does not affect the equilibrium partitioning itself. If partition constants could only be measured at equal phase volumes, this would be inconsistent with standard experimental practice and with the large body of published partitioning data, which are determined using a variety of phase volume ratios to cover a wide range of partition constants. The OECD Test Guideline 107 for measuring log $K_{ow}$ with the slow stirring method (OECD, 1995) explicitly recommends three runs with different volume ratios of n-octanol to water.

**Statement:** Fish exposed to pollutants in large (relative to the fish volume) volumes of water will take up these pollutants until their saturation concentration (maximum lipid solubility) is reached, irrespective of the pollutant's concentration in the exposure water.

**Comment:** This statement contradicts thermodynamic principles and decades of experimental experience. The established definition of steady state is a dynamic balance between uptake and elimination. During accumulation, the elimination rate increases with increasing internal concentration, reducing net uptake until a point is reached where uptake and elimination rates are equal and steady state is achieved. In standard BCF experiments conducted according to OECD Test Guideline 305 (OECD, 2012), exposure concentrations are maintained well below water solubility. Under these conditions, concentrations in the organisms will approach steady state concentrations while staying below the maximum solubility limit.

Also, a simple plausibility check directly invalidates this notion. According to it, all fish would eventually reach lipid saturation if exposed to the substance for long enough. This would imply that even extremely low environmental concentrations would lead to unavoidable toxicity, as baseline toxic effects would be reached in all fish once the lipid phase is "full."

**Statement:** Rate constants are not constant.

**Comment:** It is unclear how these time- and concentration-dependent rate constants are implemented mathematically, or what mechanistic basis would justify treating constants as variables. In particular, it is not evident why an external water concentration differing by a factor of 100 would result in only a threefold difference in internal concentration during the presumed linear uptake phase (Figure S6 in the SI). This element of the paper is fundamentally unclear.

**Statement:** The BCF is time-dependent. Longer exposure times inevitably lead to gradually higher BCFs.

**Comment:** This statement contradicts the definition of the BCF as a steady state parameter and violates the basics of thermodynamics. The observed empirical correlation between exposure time and BCF is more plausibly explained by study design. Substances with higher hydrophobicity, and often higher BCF values, generally require longer periods to reach steady state, which is why longer exposure times are typically employed for such substances. In addition, variations in organism lipid content may influence the observed BCF values and the time required to reach steady state. This may contribute to the observed correlation if normalization to a standard lipid content of 5% is not consistently applied. Apparent differences for the same compound across studies may result from measurements conducted without reaching steady state. This, however, is best interpreted as an experimental artefact rather than a conceptual flaw in the BCF itself.

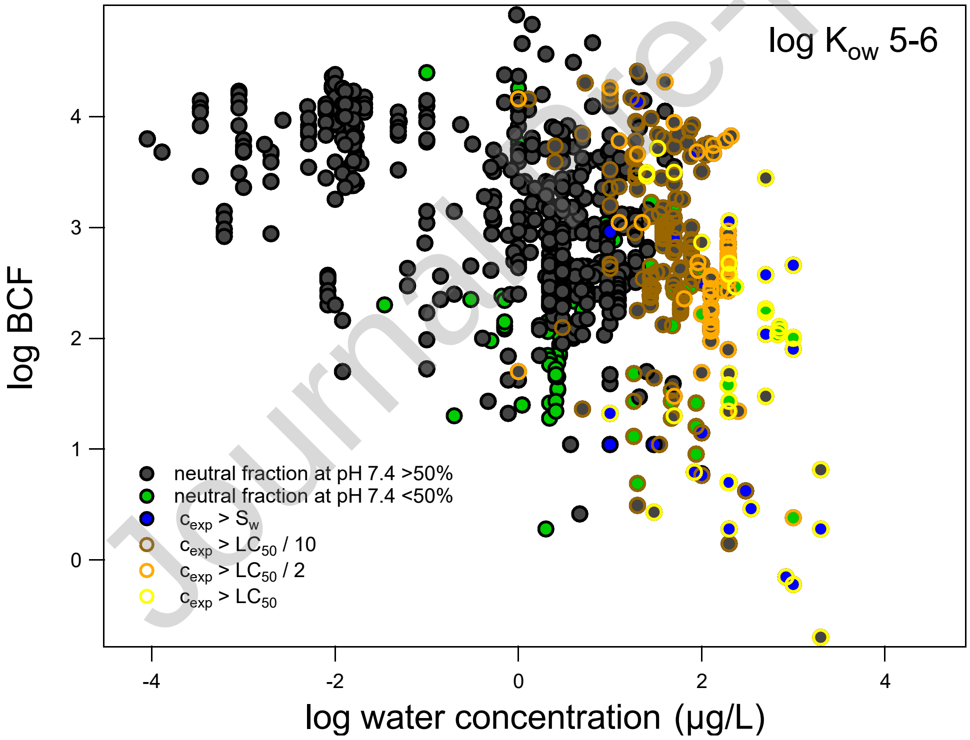
**Statement:** The BCF is concentration dependent and this can be shown mathematically.

**Comment:** A proof (mathematical derivation) is lacking and the reported empirical dependence of BCF on the applied concentration (Figure 1) is misinterpreted: To rule out the influence of hydrophobicity, the authors examined compounds within small log $K_{ow}$ ranges (Figure 1b) and still observed a significant correlation between log BCF and the applied concentration, albeit extremely weak ($r^2 = 0.16–0.21$ across the four log $K_{ow}$ ranges between 3 and 7, as reanalyzed here).

The argument suffers from a conceptual flaw: it attempts to explain what is likely an artefact of experimental design using a purely theoretical framework. In practice, experimental design is not independent of expected outcomes but is often guided by prior scoping experiments and feasibility constraints. Higher exposure concentrations are routinely selected when low BCF values are anticipated (e.g., due to biotransformation) to ensure analytical detectability. Similarly, extended exposure durations are chosen when slow uptake kinetics or delayed steady-state conditions are expected, see comment above.

These adaptive design choices introduce systematic biases that can generate apparent concentration- or time-dependencies in BCF. Such patterns therefore do not necessarily reflect underlying mechanistic behavior, but may instead arise as predictable consequences of non-random, expectation-driven experimental design.

Furthermore, the dataset used by the authors appears insufficiently curated. It includes measurements obtained at toxic concentrations, above water solubility limits, and for compounds that are predominantly ionized under physiological conditions. These factors likely contributed to the reported empirical correlation (see Figure below for the log $K_{ow}$ range 5-6 where we flagged compounds measured at toxic concentrations or concentrations above water solubility, as well as potentially charged compounds).



Experimental log BCF versus log water concentration for compounds with log $K_{ow}$ between 5 and 6 (as in Figure 1b, similar trends were observed in other log $K_{ow}$ ranges). Data were obtained from the Supporting Information Dataset_xgboost_final, using predicted log $K_{ow}$, lethal concentration 50% $LC_{50}$, and water solubility $S_w$ as reported in the SI and incorporated into the final model. Compounds predicted to have a neutral fraction below 50% at pH 7.4 (i.e., mostly ionized) were flagged using ACD/Percepta. Experimental concentrations exceeding water solubility or $LC_{50}$ thresholds (>$LC_{50}$, >$LC_{50}$/2, and > $LC_{50}$/10, where toxic effects are expected) are also indicated.

The correlation will additionally be affected by unaccounted variables, such as variations in fish lipid content, lack of growth dilution corrections, analytical challenges, the induction of metabolic processes (OECD, 2012), and uncertainties in the prediction of water solubility and toxicity.

To rigorously assess a potential concentration dependence of the BCF, a fully curated dataset would be required. Moreover, concentration-dependence of the BCF cannot be shown across different chemicals, but rather would have to be shown for individual chemicals dosed at different concentrations.

**Statement:** A large percentage of chemicals reported to EU authorities with BCF < 1000 or 2000 (and not classified as bioaccumulative) are predicted by the AI model to exceed these thresholds and are thus misclassified.

**Comment**: While it cannot be excluded that some experimentally reported BCF values are underestimating the true BCF due to methodological limitations (e.g. failure to reach steady state, testing at toxic concentrations, or exceeding water solubility limits), it remains unclear how robustly the presented AI model extrapolates to truly independent data.

From the provided "Data for xgboost" Excel file, the training set comprises 3,032 data points and the test set 760 data points. However, only 16 test compounds are not represented in the training set by at least one measurement of the same molecule, which points towards severe data leakage. Moreover, none of these 16 cases involve measurements under different exposure concentrations. Under such conditions, performance metrics may not fully capture potential overfitting of the model. This raises concerns regarding the extent to which the reported predictive accuracy reflects true extrapolation to unseen compounds.

**Further comment:**

Equation 3 of the manuscript neglects the influence of the fat compartment on the aqueous fish compartment over time. There is no term accounting for the transfer of chemical from fat back into the aqueous fish compartment ($k_4$*$C_{fl}$), nor is the loss from the aqueous compartment to fat ($k_3$*$C_{fa}$) included. Equation 5 assumes a simple relation between fat concentration and the external water concentration via $k_3/k_4$ at steady state. However, since $k_3/k_4 = C_{fl}/C_{fa}$ this relationship is only strictly valid if the aqueous compartment concentration equals the external water concentration, which would require $k_{1'} = k_{2'}$.

A corrected formulation would include the aqueous–fat exchange (corrections in red):

$$\frac{dC_{fa}}{dt} = k_{1'} * C_w - (k_{2'} + k_3) * C_{fa} + k_4 * C_{fl} \quad 3$$

$$\frac{dC_{fl}}{dt} = k_3 * C_{fa} - k_4 * C_{fl} \quad 4$$

$$BCF = (1 - BF) * \frac{k_{1'}}{k_{2'}} + BF * \frac{k_{1'}}{k_{2'}} * \frac{k_3}{k_4} \quad 5$$

**Conclusion:**

In conclusion, the authors incorrectly interpret the duration- and concentration-dependence of empirical BCF values revealed by a machine-learning model. These are not causal relationships that would require new conceptual ideas of the mechanism of bioconcentration. The suggested theory is incompatible with established thermodynamic principles governing equilibrium partitioning and the vast experimental evidence supporting these principles. Instead, these pseudo-dependencies can be explained by (i) experimental choices, e.g. influenced by analytical method sensitivity, (ii) the failure to systematically address or convincingly exclude sources of bias and error, (iii) insufficient data curation, and (iv) a dataset that is too small for reliable machine learning. Accordingly, the suggestion that there are large numbers of regulatory misclassifications of chemicals or that there is a need for new bioconcentration metrics for chemical classification is without merit.

**Acknowledgements:**

We thank James Armitage, Jon Arnot, Katrine Borgå, Satoshi Endo, Frank Gobas, Jung-Hwan Kwon, Derek Muir and John Nichols for carefully reading the manuscript and for supporting this comment.

**References:**

Köhler, H.R., Aalizadeh, R., Treu, G., Gräff, T., Peschke, K., Prutz, I., Thomaidis, N.S., Triebskorn, R., von der Ohe, P.C., 2026. By integrating previously overlooked drivers AI boosts bioaccumulation assessment in fish. J. Hazard. Mater. 501. https://doi.org/10.1016/j.jhazmat.2025.140648

OECD, 2012. Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure, OECD Guidelines for the Testing of Chemicals, Section 3. OECD. https://doi.org/10.1787/9789264185296-en

OECD, 1995. Test No. 107: Partition Coefficient (n-octanol/water): Shake Flask Method, OECD Guidelines for the Testing of Chemicals, Section 1. OECD Publ. Paris. https://doi.org/https://doi.org/10.1787/9789264069626-en

## Declaration of Competing Interest

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: