A Theoretical Protein Surface-Binding Model for Toxicokinetic Predictions in Aquatic Species

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1. Background

Understanding the relationship between external exposure and internal concentration is fundamental to environmental safety assessment [1]. A key measure of chemical accumulation in aquatic species is the ratio between the organism's internal concentration and the external water concentration. However, these ratios are commonly estimated using semi-empirical models calibrated to *in vivo* data, which limits their applicability to novel chemicals. At the same time, regulatory and societal drivers aligned with the 3Rs increasingly motivate the development of New Approach Methodologies (NAMs) that reduce reliance on animal testing [2]. Existing mechanistic bioaccumulation models predominantly emphasise lipid partitioning [3], despite evidence from pharmacokinetics and experimental studies that protein binding can substantially influence internal concentrations. External concentration–dependent protein binding has been observed but is not represented in current aquatic toxicokinetic models [4].

2. Aims & Objectives

The overall aim of this study was to develop a novel toxicokinetic model for chemical partitioning including lipids and surface-binding to proteins to determine steady-state chemical concentration ratios for a range of aquatic species and chemistries.

This can be divided into two main objectives:

1. Develop a theoretical framework for modelling lipid partitioning and protein chemical surface-binding
2. Simulate conservative steady-state concentration ratios and evaluate their performance on historical *in vivo* measurements

3. Methods

Compartmental model

An aquatic organism was represented as three internal compartments: an aqueous fraction (i), a lipid fraction (ℓ), and a protein fraction (p), exchanging chemical mass with the external water (e) (Figure 1). Uptake from the environment and elimination from the organism were assumed to occur via the aqueous compartment. Lipid partitioning and protein binding were treated as independent processes, with no direct mass transfer between lipid and protein compartments. Under long-exposure conditions, steady state was assumed such that the internal aqueous concentration satisfies $y_i = y_e$.

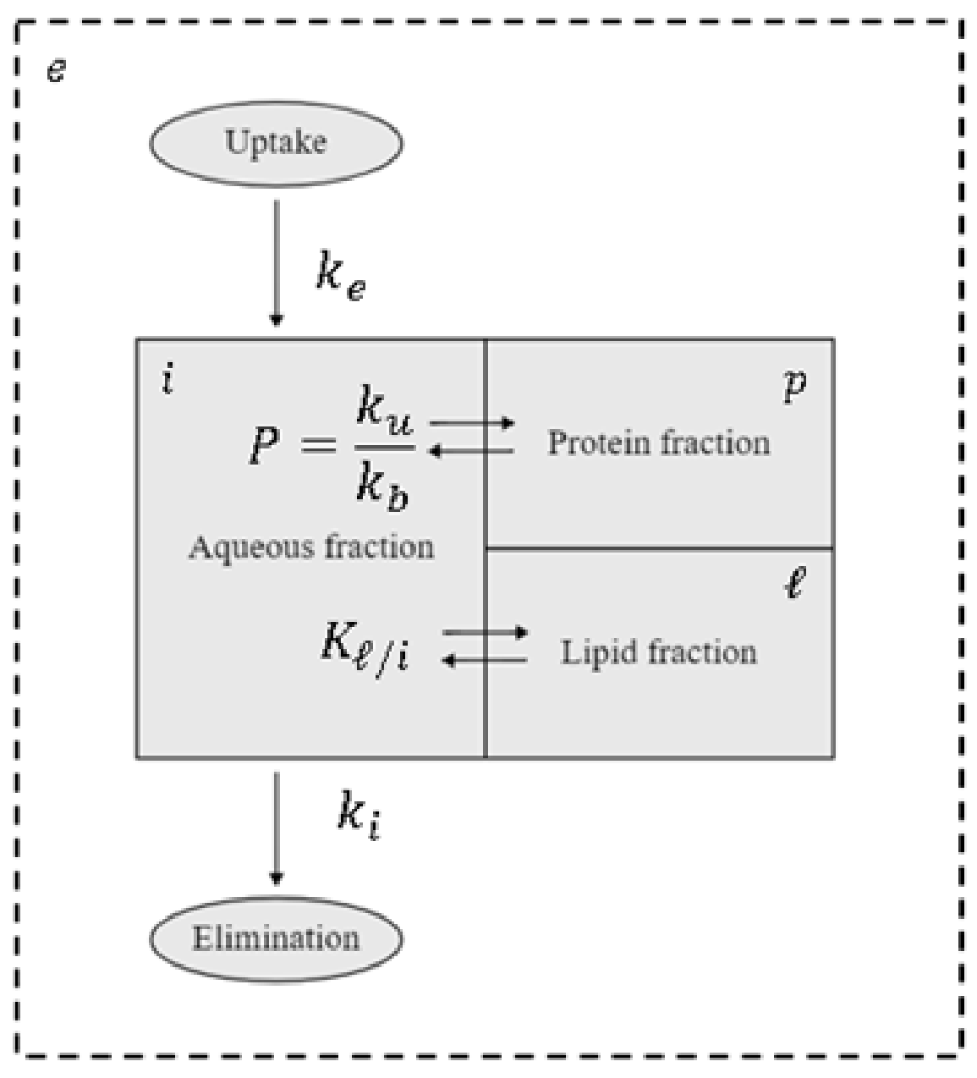

Figure 1. Compartmental model of an aquatic organism living in a body of water. The organism is composed of lipid, protein and water fractions. The protein/aqueous fraction and lipid/aqueous fraction mass transfer processes are governed by the protein affinity (P) and lipid-water partition coefficient ($K_{\ell/i}$), respectively. The uptake from the surrounding environment (external water concentration) and elimination from the internal water partition of the organism are governed by uptake (K_e) and elimination (K_i) rates.

General steady-state expression

The total steady-state internal concentration was defined as the sum of aqueous, lipid, and protein contributions, with the steady-state concentration ratio given by $z = y/y_e$. Combining lipid partitioning with a novel protein surface-binding yields the general expression,

$$z(K_{\ell/i}, y_i) = Ay_i^B + CK_{l/i} + D \approx Ay_e^B + CK_{o/w} + D,$$

where the term Ay_e^B represents protein surface-binding and $CK_{o/w} + D$ represents lipid partitioning. The parameters A and B have physical interpretations related to chemical availability and protein surface characteristics and can be estimated by calibration to toxicokinetic data spanning multiple external concentrations.

Special-case expression

A calibration-free special-case expression was derived for data-poor scenarios by assuming maximal protein binding capacity and availability. This yields,

$$z(K_{\ell/i}, y_i) = \kappa y_i^{-1/3} + \nu_\ell K_{\ell/i} + 1 - \nu_\ell - \nu_p,$$

where ν_ℓ and ν_p are the lipid and protein mass fractions, respectively, and κ is a constant determined by protein surface area, organism mass, and protein binding affinity. Protein affinity (P) was estimated using human plasma fraction unbound data (ξ), with $P \approx (1-\xi)/\xi$. This formulation provides conservative steady-state concentration ratio estimates without calibration to *in vivo* toxicokinetic data.

Data collation and model evaluation

The general and special-case expressions were evaluated using three datasets:

1. Thiacloprid exposure in *Gammarus pulex* with external concentrations spanning several orders of magnitude [4].
2. Arnot & Gobas database containing 1,973 steady-state concentration ratios across 50 aquatic species and 448 chemicals covering biocides, industrial chemicals, consumer products and persistent organic pollutants [3]. Key assumption was that protein fraction (ν_p) = $1 - \nu_\ell$.
3. AquaTK dataset for *Daphnia magna* containing high-quality time-course TK data covering 25 unique chemicals including biocides, pharmaceuticals, industrial chemicals, and PFAS [5].

4. Results

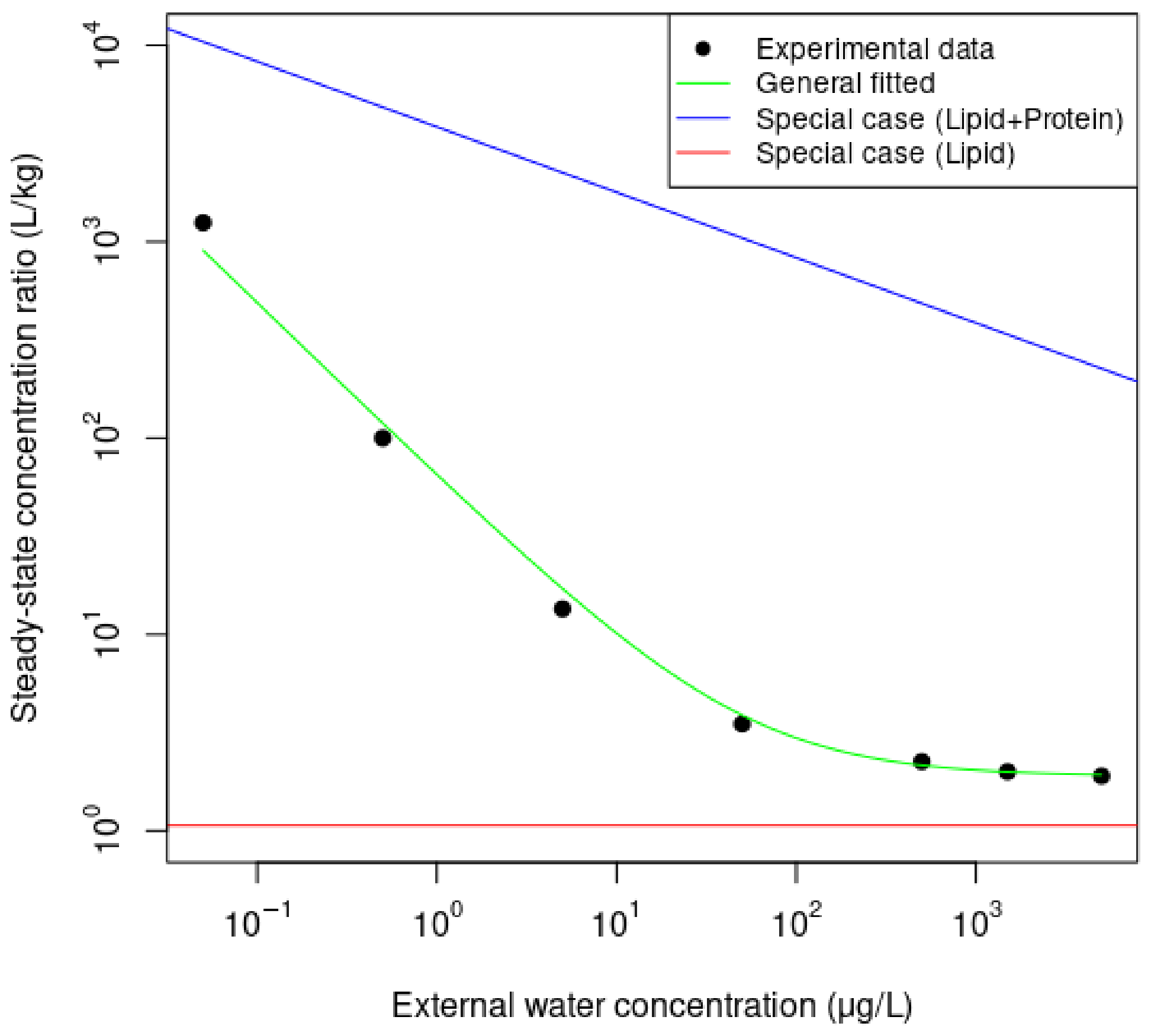

Figure 2. Thiacloprid steady-state concentration ratios over a range of external concentrations measured in *Gammarus pulex* from Raths et al. [4] compared to the general expression (black solid line), special case with the lipid and protein contributions (blue solid line), and the special case with only the lipid contribution (red solid line).

Experimental steady-state concentration ratios for thiacloprid in *Gammarus pulex* decreased markedly with increasing external concentration. The fitted general expression reproduced this behaviour, whereas the special-case expression provided conservative estimates and lipid-only predictions substantially underestimated accumulation. Fitting the general expression yielded physically interpretable protein surface-binding parameters ($A = 63.4, B = -0.88$), corresponding to ~7% effective protein binding and ~18% available surface-binding area across organism scales. The general expression accurately described accumulation across all concentrations, while the special-case expression represented a conservative upper bound where lipid partitioning is small.

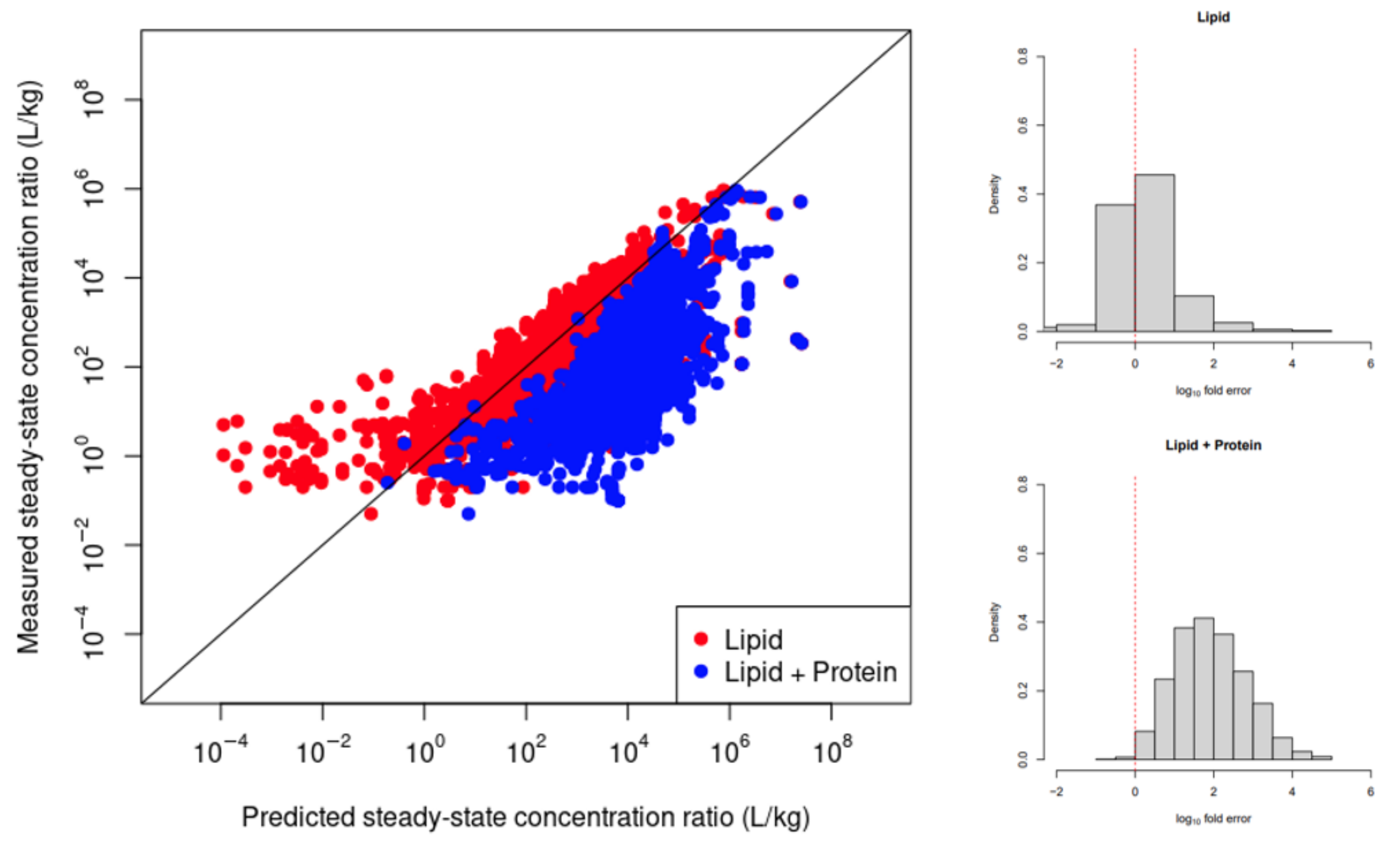

Figure 3. Left: Experimental steady-state concentration ratios from the Arnot & Gobas database [3] plotted against the predicted steady-state concentration ratios of the lipid contribution only (red dots) and the combined contribution of the lipid and protein surface-binding processes (blue dots) for 1,973 steady-state concentration ratios across 50 species and 448 unique chemicals. Right: Histograms of the log10 fold errors of the lipid contribution (top) and the lipid and protein contributions (bottom) with a red dotted line at 0 to highlight the over and under predictions of the model predictions.

The 1,973 experimental steady-state concentration ratios from Arnot & Gobas [3] were plotted against predicted steady-state concentration ratios for the lipid contribution only and for the combined lipid and protein surface-binding contributions (Figure 3). Including the protein surface-binding process resulted in a shift towards more conservative predictions. Lipid-only predictions were conservative in 59% of cases, whereas the combined lipid and protein contribution resulted in conservative predictions in 99% of cases, indicating that protein binding accounts for a substantial proportion of chemical accumulation. Histograms of $\log_{10}$ fold errors show that lipid-only predictions exhibit considerable under- and over-prediction, with a median fold error of 1.45. As $K_{o/w}$ decreases, the lipid-only expression increasingly underestimates steady-state concentration ratios, as predicted values tend towards the lipid fraction. Including protein surface-binding shifts fold errors towards over-prediction, with a median fold error of 70.15, reflecting the conservative nature of the combined expression

Using an independent high-quality *Daphnia magna* toxicokinetic dataset (AquaTK), experimental steady-state concentration ratios for 25 unique chemicals (35 steady-state ratios) were compared with model predictions (Figure 4). Lipid-only predictions were conservative in 46% of cases, whereas inclusion of protein surface-binding resulted in conservative predictions for 100% of steady-state ratios assessed. Similar levels of overprediction were observed compared with the cross-species dataset, indicating that model conservatism is not driven by assumptions on protein fraction alone. This independent evaluation confirms the generality of the protein surface-binding contribution and demonstrates the transferability of the special-case expression across datasets and species.

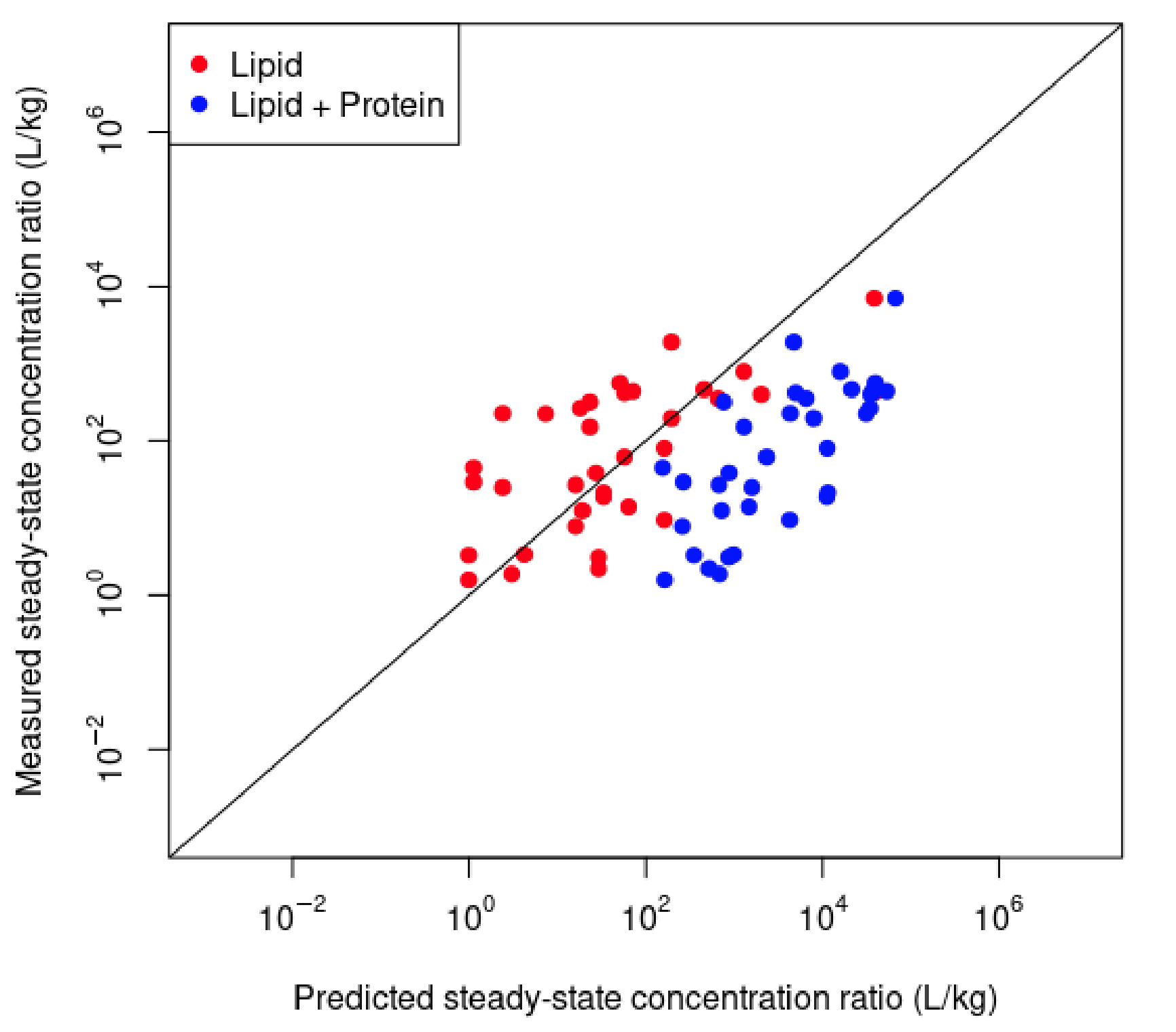

Figure 4. Experimental steady-state concentration ratios for *Daphnia magna* from the AquaTK R package (Collins et al. [5]) plotted against predicted ratios using lipid-only (red) and combined lipid + protein surface-binding (blue) contributions for 35 steady-state concentration ratios covering 25 unique chemicals

5. Take Home Messages

- Protein surface-binding is a major driver of chemical accumulation in aquatic species and explains concentration-dependent behaviour not captured by lipid-only toxicokinetic models.
- The general mechanistic expression reproduces observed steady-state concentration ratios and yields physically interpretable parameters describing protein binding and surface availability.
- A calibration-free special-case expression provides conservative predictions in 99% of cases across species and chemical space, supporting early-tier NAM-based environmental risk assessment without *in vivo* data.
- Future work will reduce conservatism by integrating additional processes such as biotransformation, while retaining the mechanistic protein surface-binding framework.

References

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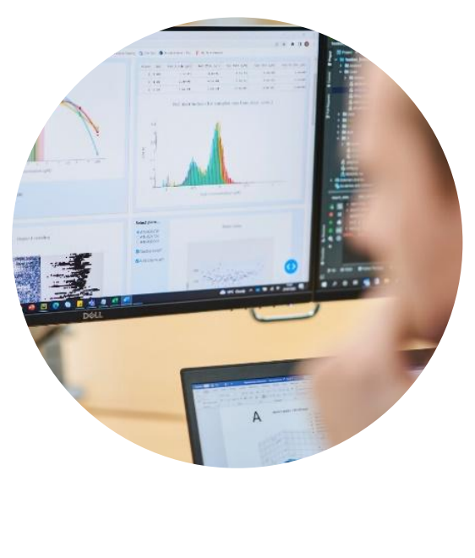

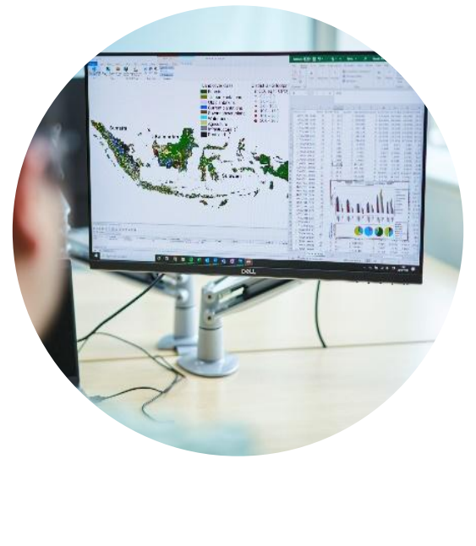

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