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Original Article

Hazard/Risk Assessment

Dietary kinetics of a PFAS mixture in the American toad (*Anaxyrus americanus*): laboratory insights into trophic transfer of PFAS

Andrew G. East[1,2,*], Mike Simini[3], Emily E. Stricklin[3], Guilherme R. Lotufo[4], Jennifer L. Guelfo[5], Zhao Yang[5], Travis Gallo[2], Michael J. Quinn[1], and Roman G. Kuperman[3]

[1]Toxicology Directorate, Defense Health Agency—Public Health, Aberdeen Proving Ground, MD, United States
[2]Environmental Science and Technology Department, University of Maryland College Park, College Park, MD, United States
[3]Research and Operations Directorate, DEVCOM Chemical Biological Center, U.S. Army Combat Capabilities Development Command, Aberdeen Proving Ground, MD, United States
[4]Engineer Research and Development Center, U.S. Army Corps of Engineers, Vicksburg, MS, United States
[5]Civil, Environmental, and Construction Engineering Department, Texas Tech University, Lubbock, TX, United States

*Corresponding author: Andrew G. East. Email: aeast@umd.edu; andrew.g.east.civ@health.mil

Abstract

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous in environmental media and are a concern for food web–driven exposure to ecological receptors. Terrestrial life stage amphibians concurrently represent taxa that have high potential for exposure but are generally data-poor in comparison to their aquatic life stages. Adult American toads (*Anaxyrus americanus*) likely have high dermal exposure to soil and eat terrestrial organisms that are likely to accumulate chemicals from soil. To better understand the relationship between dietary PFAS and toads in a trophic transfer context, toads were fed earthworms (*Eisenia andrei*) exposed to PFAS-spiked soil for 28 days and then were fed clean earthworms for 28 days—a 28-day uptake phase and 28-day elimination phase. Toad blood, liver, and remaining tissues were sampled weekly. Concentrations of PFAS were quantified in soil, earthworm diet, and toad tissues. Toxicokinetics of PFAS in toad livers, remainder, and estimated whole animal were evaluated using the methods of Organisation for Economic Co-operation and Development Test Guideline #305, a nonlinear regression approach, and a physiologically-based method. Definitive models were selected via a leave-one-out cross validation method and model parameters were used to determine kinetic trophic transfer coefficients (TTCs). Our TTC approach indicates perfluorooctane sulfonate, perfluoroundecanoic acid, and perfluorodecanoate are likely to magnify and 8:2 fluorotelomer sulfonate and perfluoroheptane sulfonic acid are likely to transfer or dilute in the worm-toad transition. Most PFAS have similar uptake rates, but elimination rates are clustered, suggesting that kinetics are driven by elimination mechanisms. These laboratory data use field-representative exposure approaches and provide inference about internal kinetics of individual PFAS as well as the potential for trophic transfer from soil invertebrates to terrestrial life stage amphibian predators.

Keywords: toxicokinetics, biomagnification, mixtures, ecological risk assessment

Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic molecules defined by durable carbon-fluorine polar covalent bonds generally considered ubiquitous in environmental media. One of the foundational works in PFAS observations in wildlife is based on samples collected globally, with indications that higher trophic level organisms' tissue concentrations were likely the function of trophic magnification and bioaccumulative processes (Giesy & Kannan, 2001). Early observations in aquatic systems in the field (i.e., Giesy & Kannan, 2001) were supported with laboratory work on dietary accumulation and aquatic bioconcentration of PFAS (perfluorooctane sulfonate [PFOS]) in fish and amphibians (Ankley et al., 2004; Martin et al., 2003a, 2003b). Observations of PFAS in terrestrial organisms in the field (i.e., Giesy & Kannan, 2001) were supported by laboratory observations of dietary accumulation (Newsted et al., 2006). More recent work has identified a number of field and laboratory observations of accumulative properties across a broadening range of PFAS (see review of Evich et al. [2022]).

Amphibians, as a group of organisms, are generally considered to be understudied in ecotoxicology. Regarding PFAS and amphibians, there is a substantial amount of data and information about a few PFAS in larval amphibians. Ankley et al. (2004) performed the first exposures of tadpoles to PFOS and observed toxicological effects and quantified accumulation of PFOS from water into tadpole tissues. Since that study, the combined works of Strategic Environmental Research and Development Program study ER-2626, "Development of amphibian PRAS TRVs for use in ecological risk assessment at AFFF sites" have resulted in four PFAS (PFOS, perfluorooctanoic acid [PFOA], perfluorohexane sulfonate [PFHxS], and 6:2 fluorotelomer sulfonate [6:2 FTS]) in nine amphibian test species in two experimental settings and four

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exposure routes. Larval amphibian toxicity and accumulation data from these studies is largely used to inform aquatic toxicity. However, as described by Flynn et al. (2021), some amphibian taxa spend substantial portions of their life in terrestrial life stages. To address this concern, Flynn et al. (2021) exposed post-metamorphic (terrestrial life stage) salamanders (*Ambystoma tigrinum*) to PFOS, PFOA, PFHxS, and 6:2 FTS via diet (crickets fed spiked food and water) for 30 days. Evidence for biomagnification is reported as "limited," but PFOS biomagnification factors (BMFs) are reported to be between 1.01 to 3.04 negatively correlated with diet concentration (Flynn et al., 2021). Biomagnification factor values for PFOA, PFHxS, and 6:2 FTS are all less than 1 (range = <0.001–0.072) suggesting that there is little accumulation of these PFAS in terrestrial life stage amphibians exposed via diet (Flynn et al., 2021).

In the interest of expanding available exposure data in terrestrial life stage amphibians in a time-efficient manner while retaining some ties to potentially mechanistic factors, a balance of complexity and resolution is needed. For instance, utilizing a relatively large range of PFAS that are observed at field sites (East et al., 2025) and exposure in a food web context (via diet). Data collected in such a manner could inform screening data (Zodrow et al., 2021), of which terrestrial life stage amphibians remain a data gap. Further, given the dominance of terrestrial life stages in the lifespan of some amphibians, internal kinetics are likely highly informative. Toads may not reach sexual maturity until several years of age (Willson et al., 2012; Willson & Hopkins, 2013); this implies that any site with a stable population likely has toads that may experience years of exposure. Most studies on PFAS kinetics are focused on high resolution, so based on logistical constraints, they require direct dosing of single PFAS (e.g., intravenous). In contrast, fully field observational studies of food web transfer (e.g., Huang et al., 2022; Müller et al., 2011) do not generally consider internal kinetics, taxa life-history traits, or other spatiotemporally relevant factors. Therefore, whereas a field study may be highly physicochemically mechanistic and detailed (Fremlin et al., 2023), their applicability in a spatiotemporally explicit, individual-based scenario remains untested; that is, we cannot confidently include or exclude characteristics such as animal age or length of exposure as influential factors in PFAS exposure.

Accordingly, there is a need for manipulative studies of moderate complexity. Causal inference requires an experimental approach (i.e., laboratory study; Anderson, 2008), but some ties to information to support ecological risk assessment (complexity of PFAS mixture, exposure via diet) are needed. To attempt to address these issues, a series of studies have been performed where a large number of PFAS were spiked at a uniform nominal concentration in soil. Then plants and worms were independently grown in spiked soil and the plants were then fed to rabbits and worms fed to toads (Kuperman et al., 2025) This work addresses the dietary exposure of toads to PFAS in their diet, where, critically, the dietary concentrations emerge from the processes that lead to PFAS accumulation into worms (described in Lotufo et al., 2025). Accordingly, the approach in this study controls for diverse media concentrations seen in field sites and is specifically focused on the actual relationship in question—the toad and the PFAS—in isolation from other ecological/behavioral factors that may introduce variability observed at field sites.

To utilize these insights in an ecological risk assessment exposure estimate/characterization, we need to ensure that we can speak to common food web/exposure factor models (U. S. Environmental Protection Agency [USEPA], 1993; Zodrow et al., 2021). These approaches are largely based on multiplicative factors: soil concentration times bioaccumulation factor equals worm concentration; worm concentration times biomagnification factor equals toad concentration, and so on. Because PFAS have highly variable kinetics, it is challenging to assert that steady state/dynamic equilibrium tissue concentrations have been reached in a laboratory setting with a PFAS mixture. To address this issue, we provide kinetic parameter-based trophic transfer coefficients (TTCs) that are intended to be analogous to trophic magnification factors. The premise is that inclusion of kinetics captures time-dependent processes that may be influential on field observations.

More specifically, the objectives of this study were to (1) quantify the internal concentrations of PFAS in terrestrial amphibians exposed via diet to a mixture of thirteen PFAS and (2) use model parameters to inform the sense of trophic transfer in a soil-invertebrate-predator food chain. Few field data exist in these taxa, none account for internal kinetics, and laboratory control on external factors allows for evaluation of the actual relationship of interest in ecological risk assessment—the PFAS to animal relationship. This study was part of a larger effort (Kuperman et al., 2025) to understand individually, and as a class, the movement of PFAS from soil into worms, PFAS elimination from worms (Lotufo et al., 2025), and, here, the movement of PFAS from worms to toads and toads' elimination of PFAS. The closing insight around trophic transfer is intended to support food web–based exposure estimates where data from the field may be lacking or highly variable or unclearly associated with explanatory factors.

Methods

PFAS selection

The PFAS selection is described in detail in Kuperman et al. (2025). In brief, the PFAS selected for this study included those listed in the USEPA's third Unregulated Contaminant Monitoring Rule: perfluoroheptanoate, PFOA, perfluorononanoate (PFNA), perfluorobutanesulfonic acid, PFHxS, and PFOS. To explore the effects of chain length, we included perfluorobutanoate, perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), and perfluorodecanoate (PFDA). Precursors 8:2 FTS and perfluorooctane sulfonamide were also included. The selected list captures chain length trends and the terminal transformation products of PFAS precursors and PFAS that are a primary focus of federal advisories and state regulations found on United States Department of Defense (DoD) installations. Analytical-grade PFAS were obtained from the USEPA PFAS Chemical Library or Sigma-Aldrich (St Louis, MO).

Test soil

The test soil conditions are described in detail in Kuperman et al. (2025). In brief, test soil was Organisation for Economic Co-operation and Development (OECD; Paris, France) standard artificial soil (SAS) modified by lowering the peat content from 10 to 5% (75% fine sand, 20% kaolin clay, 5% finely ground sphagnum peat moss, and 1% pulverized lime) to increase bioavailability of the test compounds (OECD, 2010, 2012, 2016). The measured concentrations of background PFAS in SAS were low (0.07 ng/g and 0.09 ng/g for PFHxA and PFPeA), with no other PFAS compounds found above detection limits. Prior to the addition of earthworms, SAS (PFAS-spiked and control) were put through an aging process for 14 days that included wetting, drying, and mixing the soil one time each week.

Diet preparation

Thirteen PFAS (see online supplementary material Table S1) with earthworm bioaccumulation factor (BAF) ≥ 2 observed in preliminary studies of the PFAS described above (Kuperman et al., 2025) were spiked into the soil used to generate the toad diet earthworms. Solutions of selected PFAS in ASTM Type I water (18 MΩ deionized water) were individually added to one batch of soil to produce 0.1 mg/kg of each PFAS in soil as a uniform nominal mixture (approximately 1.3 mg/kg ΣPFAS). This PFAS mixture spiked soil was then used to expose the earthworms, which were subsequently used to feed the toads. Control soil was developed by wetting SAS with comparable volumes of ASTM Type I water. See Kuperman et al. (2025) for more details.

After aging spiked and unamended SAS, earthworms (*Eisenia andrei*) were exposed in each soil for 28 days. On collection, earthworms were rinsed with ASTM Type I water, counted, weighed en masse, placed in 800-ml glass jars, and kept at 4°C in the dark. Less than 24 hr after collection, earthworms were blended in batches, homogenized by mixing and blending batches, dispensed into aliquots in 50-ml polypropylene conical vials, and frozen at –80°C. A subsample of thawed earthworm diet (blended, homogenized earthworm tissue) was retained for development of dosing standard curve (below).

American toads (*Anaxyrus americanus*) care, dosing, and sample collection

This animal use was reviewed and approved by the Defense Centers for Public Health-Aberdeen (DCPH-A) Institutional Animal Care and Use Committee (DCPH-A IACUC Protocol #: 06-22-02-02). The animal facility at DCPH-A is fully accredited by AALAC International, and all animal care and use was performed according to the Guide for the Care and Use of Laboratory Animals (National Research Council [U.S.] 2011) and all applicable federal and DoD regulations.

Toads ($n = 64$) were purchased from Carolina Biological Supply Company (Burlington, NC, USA), and are wild-caught animals from the eastern United States from unknown locations. Toads were quarantined and observed for 7 days prior to dosing to ensure all animals were eating and maintaining/gaining weight. Prior to dosing, toads were fed live crickets (Fluker Farms, Port Allen, LA, USA) every other day. Crickets from this supplier have been measured as nondetect for PFOS and PFHxS (unpublished data) but published cricket ΣPFAS concentrations (Choi et al., 2023) are >100-fold lower than toad diet, so are unlikely to be influential on toad background PFAS. Toads were housed in acrylic cages 25.4 cm × 47 cm × 15.25 cm, with 5 cm wetted coco coir, a 1-L paper cup hide, a polypropylene petri dish with water, and approximately 500 ml wetted sphagnum moss. All wetted materials were misted as needed (generally daily) using moderately hard synthetic freshwater (USEPA, 2002) and the Petri dish water was replaced daily. Hide, bedding, and Petri dishes were replaced as needed on soiling or saturation. A cage was reserved with temperature and humidity monitors to ensure cage-level parameters were within target. Room and cage level temperature (target 15.6–24.4°C) and humidity (50%–70%) were monitored and recorded daily. Light cycle was 12:12 hr on-off. Toads were weighed weekly (Tuesdays) and on their day of collection (Wednesdays).

Dosing was performed via a pseudo-gavage where a measured amount of worm homogenate from a press-fit syringe was dispensed into the back of the toads' mouths. Their mouths were held open by a blunt spatula and on release of the dose and spatula, the toads swallowed the dose. This method is motivated by the pseudo-gavage methods for liquid dosing used in lizards (Weir et al., 2023). The dose in mass of worm per mass of toad (mg/kg) was determined by a standard curve generated by weighing a range of volumes where reasonable accuracy could be expected from a 3-ml disposable press-fit syringe (see online supplementary material Figures S1 and S2 for mass per worm and mass per volume of worm homogenate). Toads were dosed in a manner that was intended to mimic natural foraging where toads would eat "a worm." Accordingly, doses were delivered at volumes accurate for the syringe but approximating one worm per day in a time weighted average manner (two worms Monday and Wednesday, three worms on Friday).

Samples were collected from toads after anesthetizing the toad in neutral-buffered MS-222 (tricaine methanesulfonate, brand name Tricaine-S) at 3 g/L and then decapitating the toad and pithing the brain. The heart was exposed and a 1-ml insulin syringe was used to exsanguinate. Whole blood was gently expelled into prelabeled 1.8 ml cryovials. The liver was then excised and placed in an uncoated prelabeled aluminum foil packet. The remainder of the organism was placed in a prelabeled 118 ml Nasco brand "Whirl-Pak" and sealed. All tissues were frozen on collection and stored in –80°C.

Study design

The study design was based on the OECD Technical Guide # 305, *Bioaccumulation in fish: Aqueous and dietary exposure* (OECD, 2012; Figure 1). Exposure was strictly through the dosed diet. Toads were randomly selected for sampling dates from Days 0, 7, 14, 21, 28, 35, 42, 49, or 56. Sixteen toads ($n = 16$) were sampled on study Day 0 and then six ($n = 6$) at each timepoint thereafter. Study Day 0 represents background concentrations, samples collected on study Days 7, 14, 21, and 28 represent the uptake period. Study Day 28 concurrently represents the start of elimination as the diet provided on Day 28 is the control diet. The elimination period samples were from study Days 28, 35, 42, 49, and 56.

Analytical determination of PFAS in diet and toads

Extended details of analytical determination of PFAS concentrations in earthworm homogenate and toad tissues (liver and remainder) are available in Kuperman et al. (2025). In short, PFAS were extracted from earthworms using a method that is based on extraction from fish tissue and earthworms (Malinsky et al., 2011; Rich et al., 2015). A small mass of dried earthworm homogenate was spiked with an extracted internal standard, acetonitrile was added, and the tube was vortexed and shaken. Tubes were then frozen (–20°C) to precipitate lipid and protein. Extracts were separated by centrifugation and transferred to glass

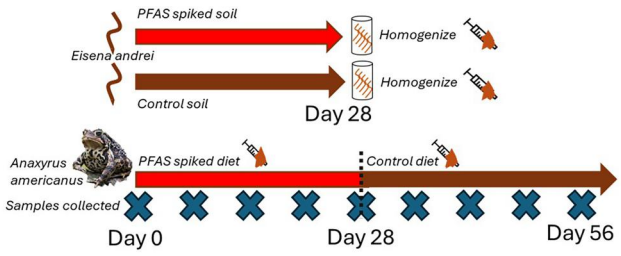

Figure 1. Description of exposure study design. Earthworms (*Eisena andrei*) were grown in one of per- and polyfluoroalkyl substance (PFAS) spiked soil or control soil for 28 days. Earthworm homogenate was prepared to dose to American toads (*Anaxyrus americanus*). Toads were dosed with spiked diet until Day 28, at which point remaining toads were fed control diet. Toad starting sample size was 64 with 16 toads sampled on Days 0 and 6 for all weekly timepoints thereafter.

scintillation vial with dilute formic acid. The extracts were then evaporated to dryness under nitrogen. Samples were reconstituted in liquid chromatography–mass spectroscopy-grade methanol and transferred to tube with ENVI-Carb. Autosampler vials were prepared for analysis with a volume of extract and volume of method and water to reach a 70:30 water-methanol solution at 200 ng/L internal standard.

The PFAS were extracted from toad liver and remainder using methods previously developed for animal tissues (Houde et al., 2008; Tomy et al., 2005; Zhao et al., 2013). A small mass of wet tissues was placed in a polypropylene tube and dried at 70 °C. An internal standard was added to each tube, methanol was added to each tube, and tubes were sonicated at room temperature. Samples were centrifuged and evaporated to dryness under nitrogen. Extracts were reconstituted in liquid chromatography–mass spectroscopy-grade methanol, transferred to a tube with ENVI-Carb, vortexed, and centrifuged. An aliquot of the extract was transferred to an autosampler vial and amended with 70:30 water-methanol to achieve a final internal standard concentration of 200 ng/L.

Chromotographic separation was performed on a Gemini C18 analytical column coupled with a Gemini C18 guard column with a Sciex Exion high-pressure liquid chromatography system. A Luna C18 delay column was installed between the mobile phase mixer and the sample injector to minimize background contamination. Columns were maintained at 40 °C throughout the run. The aqueous phase was ammonium acetate solution and the organic phase was 100% methanol. See Kuperman et al. (2025) for details on ramp schedule.

Quadrupole time-of-flight mass spectrometry (QTOF MS) for targeted analyses were performed on a Sciex X500R QTOF MS system. Turbo ion spray was used as the ion source. Multiple reaction monitoring high-resolution acquisition mode was used with two transitions (quantifier and qualifier) for each PFAS, where possible. Data were acquired and processed using versions 1.5 and 2.2 Sciex OS software. The PFAS were quantified using isotope dilution over a calibration range of 0.5–5,000 ng/L with coefficient of determination > 0.99.

Toxicokinetic modeling

Tissue concentrations (liver, remainder, and estimated whole body) were fit with several types of models, models were compared by predictive power, and the best performing model was selected to generate TTCs.

Data handling

Liver and remainder data for each toad at each timepoint across 16 PFAS were used for toxicokinetic modeling in contrast to utilizing timepoint-specific summary statistics. Nondetects observed in toads on the first timepoint (study Day 0) were set to the PFAS-specific minimum observed across the study period. This increases the stability of background parameter estimates but may lead to overestimates of background means. All other nondetect observations across toads (study Day ≥ 7) were set to NaN (not a number). Data from timepoints ≥ study Day 28 were also labeled with an elimination day, which is study Day−28.

Worm diet aliquot ($n = 5$) PFAS-specific analytical estimates were summarized to a mean and used as the "dose" parameter for the entirety of the uptake period or the whole study period per model requirements. Control diet aliquots were all nondetect and set to zero to maintain mathematical continuity during elimination period per model requirements.

Liver and remainder concentrations were used to generate an estimated whole-body concentration. The liver of a toad represents approximately 5% bodyweight (Finkler et al., 2014) and lacking the serum data, the remainder was assumed to represent 90% of the bodyweight. Accordingly, the estimated whole animal concentration is 0.05 (liver) + 0.90 (remainder). Estimated whole body concentrations are per animal and per PFAS and were only calculated for those toads with concentrations above reporting limits for both tissues; those toads with only one quantified concentration for either liver or remainder would have an NaN for estimated whole body. Reporting limits are different for these tissues and exclusion is critical to avoid highly biased estimates.

Nonlinear regression approach

Nonlinear model

The nonlinear regression approach is defined by utilizing the model of highest performance for predicting tissue (serum) concentrations of PFOS in rabbits and chickens exposed via diet (Tarazona et al., 2015, 2016). Starting parameters were determined by using linear regression techniques of OECD test guideline #305 for the elimination period and uptake period. Some modifications to the OCED test guideline #305 techniques were made during the uptake period estimation given that the "linear phase" of uptake was highly variable and the volume of distribution (Vd) was incorporated into the nonlinear models and likely captures the needed variability without forcing unrealistically high uptake rates based on Day 0 to Day 7 data alone.

The one compartment nonlinear model was used here to evaluate PFAS-specific toxicokinetics:

$$C_t = background + \left[\frac{(D \times K_{01})}{(Vd \times (K_{01} - K_{10}))}\right] \times \left[e^{-K_{10} \times t} - e^{-K_{01} \times t}\right] \quad \text{(Eq. 1)}$$

where concentration at study day (C_t) is a function of the mean PFAS-specific concentration at study Day 0 (*background*); the PFAS concentration in the diet times the time-weighted average dosing fraction ((3/7)[diet] =*D*); the volume of distribution (*Vd*); the uptake rate (K_{01}); the elimination rate (K_{10}); and time (study day, *t*).

Nonlinear model parameter estimation

All parameters were estimated using R (R Core Team, 2024) and are estimated by PFAS and tissue (liver, remainder, and estimated whole body). The first parameter estimation step was to estimate the elimination rate (K_{10}) using linear regression (least squares) of the natural log of the concentration through 28 days of elimination period (study Day 28–56 as elimination Day 0–28). The slope of that linear regression is the estimated elimination rate. The second step was to fit *Equation 1* using nonlinear least squares and Port algorithm with the nls() function in R (R Core Team, 2024) with the elimination rate set to the slope of elimination linear regression. The unknown parameters that were estimated were the uptake rate (K_{01}) and volume of distribution (*Vd*). Both K_{01} and *Vd* were bounded between 0–1 and 0–10, respectively. For PFAS where tissue concentrations were difficult to distinguish from background, a Levenberg-Marquart algorithm was used (Elzhov et al., 2023). Bounds for K_{01} and *Vd* were also expanded to 0–2 and 0–100 in these cases. Note that the Levenberg-Marquart algorithm is robust in difficult to estimate parameter situations, similar to the cases we identified where peak concentrations were difficult to differentiate from background. Parameter estimate variability was quantified using bootstrap methods (Baty et al., 2015). Residuals are resampled 999 times and least-square estimates of parameters are used to provide confidence intervals of definitive parameters estimates. Bootstrapping incorporates some of the nonparametric

characteristics of the observed data and does not assume parametric/Gaussian distributions, so is a more robust route to representing variability in parameter estimates.

The third stage of parameter estimation was to explore elimination rates in the nonlinear approach. Accordingly, a second round of fitting *Equation 1* was performed with the uptake rate set at the value identified in the first round of fitting *Equation 1*. The elimination rate (K_{10}) and Vd were estimated using the nls() or nlsLM () function as needed to reach stable parameter estimates. The parameter estimate variability is represented by the same bootstrap procedure as above to provide confidence intervals around estimates.

Ordinary differential equation system

We differentiate this model from physiologically based models as whole blood concentration nor tissue volumes were determined; subsequently, explicit transport cannot be sufficiently tracked. However, a two-compartment model is physiologically relevant as the remainder concentration is "upstream" and "downstream" of the liver concentration. The system of liver and remainder were described as two state variables and several flows. There was a dietary contribution (on a schedule, [D_i]) adjusted by an absorption factor (a) into the remainder, then fluxes into (k_{12}) and out (k_{21}) of the liver, and finally, elimination (k_{el}). These fluxes (see online supplementary material Figure S11) and their differential equation system

$$\frac{dRemainder}{dt} = aD_i + k_{21}Liver - k_{12}Remainder - k_{el}Remainder,$$
$$with\ D_i = \begin{cases} D, & i = 0,\ 2,5,7,9,12,14,16,19,21,23,26,28 \\ 0, & i = 1,3,4,6,8,10,11,13,15,17,18,20,22,24,25,27,29\ldots56 \end{cases} \quad \text{(Eq. 2)}$$

$$\frac{dLiver}{dt} = k_{12}Remainder - k_{21}Liver \quad \text{(Eq. 3)}$$

where on the actual dates of dosing (Monday, Wednesday, and Friday during the uptake period), i added to the remainder concentration is the diet concentration (D) multiplied by an absorption factor (a), and on all other timepoints, remainder concentration is a function of input from liver ($k_{21}Liver$) and flux to liver ($k_{12}Remainder$) and elimination to waste ($k_{el}Remainder$), and the liver is the balance of rates in ($k_{12}Remainder$) and out ($k_{21}Liver$).

Ordinary differential equation model parameter estimation

Parameter estimates were obtained for the system of *Equations 2* and *3* by a model cost reduction algorithm, the modcost () function in the FME package (Soetaert & Petzoldt, 2010) in R. Residual error was the quantification of model cost. Model predictions were solved via the deSolve package in R (Soetaert et al., 2010). Starting parameters were based on nonlinear model uptake and elimination rates (elimination used for both liver and remainder).

Model selection via cross-validation

As these two model types (nonlinear vs. ordinary differential equation [ODE]) have vastly differing mathematical structures, it is inappropriate to utilize common model comparison strategies such as information criterion (Akaike's information criteria) that rely on measures of model complexity and error from nested model structures. Leave-one-out cross-validation (LOOCV) is a maximal k-fold cross-validation technique where instead of a training and test dataset partitioning the dataset, each ith datapoint is used as a test dataset against a model developed from n-i data. Although many statistical descriptors of the resultant distribution of errors (ith prediction from n-i model—ith observed) are possible, here, the mean absolute error (MAE) was used.

The definitive model was selected based on lower relative MAE across liver concentrations of PFOS and 8:2 FTS. These PFAS, in these tissues, capture two extremes of concentration trajectories observed—very slow and very fast elimination. These were selected for computing efficiency and avoiding confounding interpretations from PFAS or tissues with concentrations indistinguishable from background. In summary, LOOCV provides a quantitative basis to compare models' predictive power and select the model with the best predictive power across models with no mathematical relation.

TTCs

Utilizing laboratory data to speak to trophic transfer (considering the spectrum of trophic magnification to trophic dilution [Newman, 2020]) requires ensuring careful handling of time or clear understanding of dynamic equilibrium/steady state of concentrations and fluxes. Presumably, field data are representative of dynamic equilibrium/steady state. Laboratory data are generally known to either be in a steady state or not. In these data, as PFAS were observed in both of these states at Day 28, static representations of biomagnification (C_{toad}/C_{diet}) per-PFAS from these data would be inaccurate representations of field trophic transfer observations.

A kinetic approach was utilized to account for both time and internal kinetics (i.e., Vd) using the uptake and elimination rates. In short, $TTC = Uptake/Elimination$, where *Uptake* and *Elimination* represent parameters from the definitive model selected from the LOOCV procedure. To ensure sufficient capture of observed variability (as a measure of uncertainty), bootstrap parameter estimate distributions were resampled (with replacement) 10,000 times to generate probabilistic estimates of TTCs.

Results

Smoothed trajectories of PFAS concentrations in liver, remainder, and estimated whole animal (Figures 2 and 3) show that several toxicokinetic trajectories can be expected. Whereas diet concentrations (see online supplementary material Figure S3) vary, it is clear that few of the PFAS have little uptake. Several of the PFAS have what appear to be fast elimination rates (e.g., 8:2 FTS) and several have slow elimination rates (e.g., PFOS). Importantly, the patterns across liver or remainder appear similar, but in general, liver concentrations are higher than remainder. Due to the estimation of whole body concentration approach, remainder drives the bulk of estimated whole body concentration but given "parallel" trajectories between liver and remainder, kinetics are similar across tissues.

An opening hypothesis is that internal kinetics and ultimately trophic transfer of PFAS in this diet to organism step are driven by elimination rate. There are no negative PFAS-to-PFAS correlations in the toad tissues (see online supplementary material Figure S4), so it is unlikely that kinetics are a function of transformation or degradation processes. Relationships between Day 28 toad tissue concentrations and diet are highly variable (see online supplementary material Table S2), so there is further evidence that observed concentrations are a function of kinetics.

Definitive model selection

Leave-one-out cross validation was used to differentiate model techniques based on their MAE as a measure of predictive power. Ultimately, TTCs should be determined based on the model type

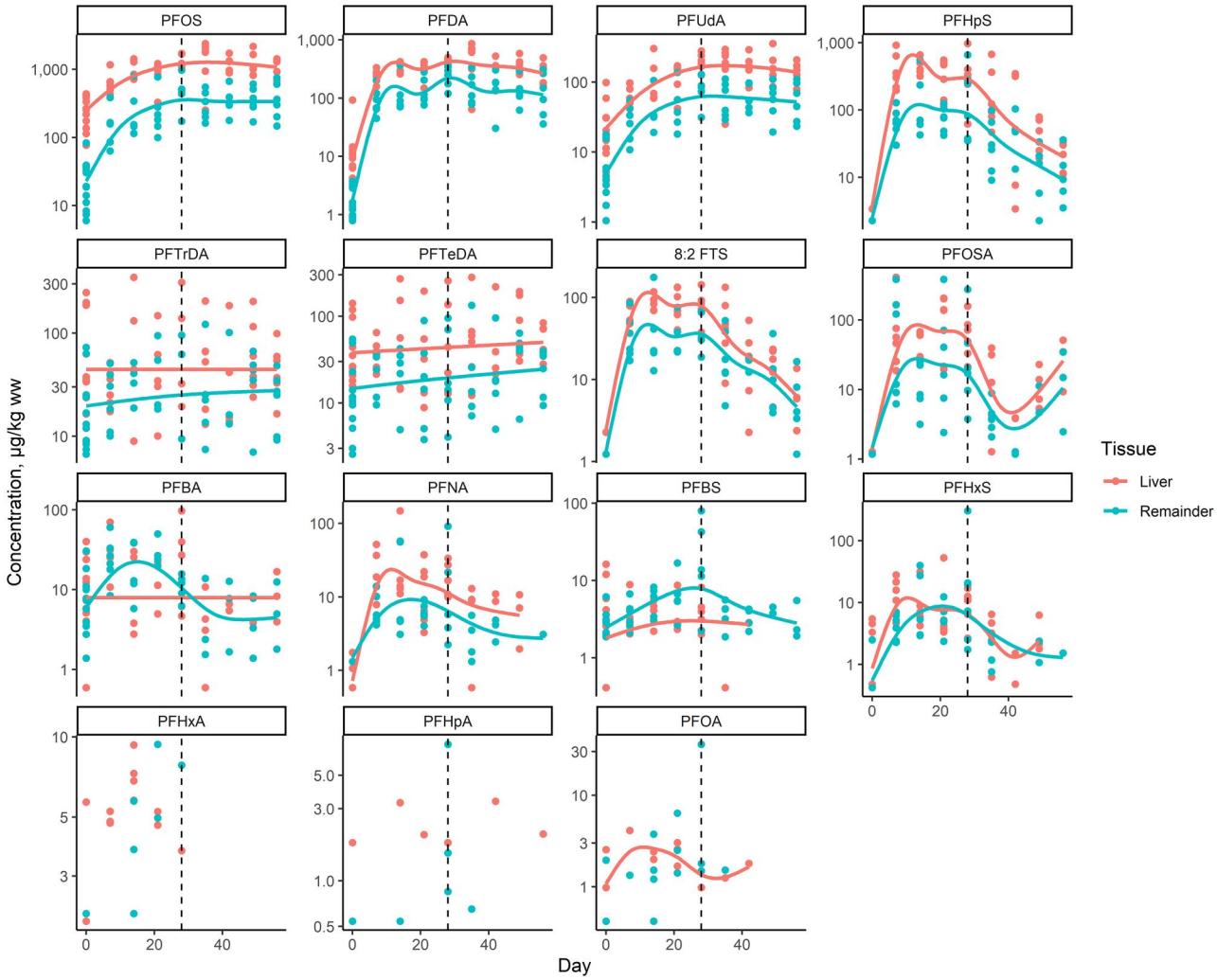

Figure 2. Liver and remainder per- and polyfluoroalkyl substance (PFAS) concentrations (µg/kg wet wt) with tissue- and PFAS-wise cubic spline smoothers. Points are individuals; lack of points indicates no data above reporting limit, dashed vertical line indicates Day 28 transition from uptake to elimination. ww = wet weight; PFHxS = perfluorohexane sulfonic acid; PFBS = perfluorobutane sulfonic acid; PFHpS = perfluoroheptane sulfonic acid; 8:2 FTS = 8:2 fluorotelomer sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFOS = perfluorooctane sulfonic acid; PFDA = perfluorodecanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluoroocanic acid; PFUdA = perfluoroundecanoic acid; PFBA = perfluorobutanoic acid; PFHpA = perfluoroheptanoic acid; PFTeDA = perfluorotetradecanoic acid; PFTrDA = perfluorotridecanoic acid; PFHxA = perfluorohexanoic acid.

with the highest performing predictive capacity. The PFOS and 8:2 FTS concentrations in liver were selected for this analysis. The ODE model type led to twofold increases in MAE over the nonlinear model type in both PFOS and 8:2 FTS liver concentration trajectories (Table 1). This suggests that the nonlinear model should be selected for definitive parameter estimation and subsequent TTC determination. Of note, regardless of the model type or chemical, there was little influence of removing individual data on MAE compared with full models (Table 1). The implications of this are that "the data fit the model" and predicting liver concentrations as single compartment flux is likely accurate to biological processes. Accordingly, TTC estimates from either model are likely equivalently accurate, but the reduced amount of error in the nonlinear model increases the precision of the TTC estimate.

Toxicokinetic parameters and analysis

Toxicokinetic parameters were estimated in a stepwise fashion using guidance from OECD test guideline # 305 and Tarazona et al. (2015, 2016). The linear regression of $\log_e$(concentration) through elimination period (study Day 28 through 56) provides a definitive elimination rate for all PFAS (Table 2). Importantly, these regression parameter estimates are of highly varying quality (see SE in Table 2 and online supplementary material Figures S5–S10). As the data may not be parametric and *Vd* may be influential, the bootstrapped nonlinear model elimination parameter estimates are likely the most appropriate representation of elimination rate. Estimates of half-life (days) are provided using these parameter estimates and their 95% confidence intervals (Table 2).

TTC estimates

The ratio of uptake and elimination here is intended to speak to the potential for trophic magnification/transfer/dilution in the diet to consumer (worm to toad) trophic step while considering internal kinetics. Less than the complete suite of PFAS were successfully modeled in the definitive nonlinear methods, so some TTCs (not definitive) from linear models (i.e., the OECD test guideline #305) were generated. Importantly, although the actual values may be inaccurate, the overarching patterns of potential for trophic magnification-transfer-dilution across PFAS appear consistent (Figure 4). Specifically, PFOS is consistently observed

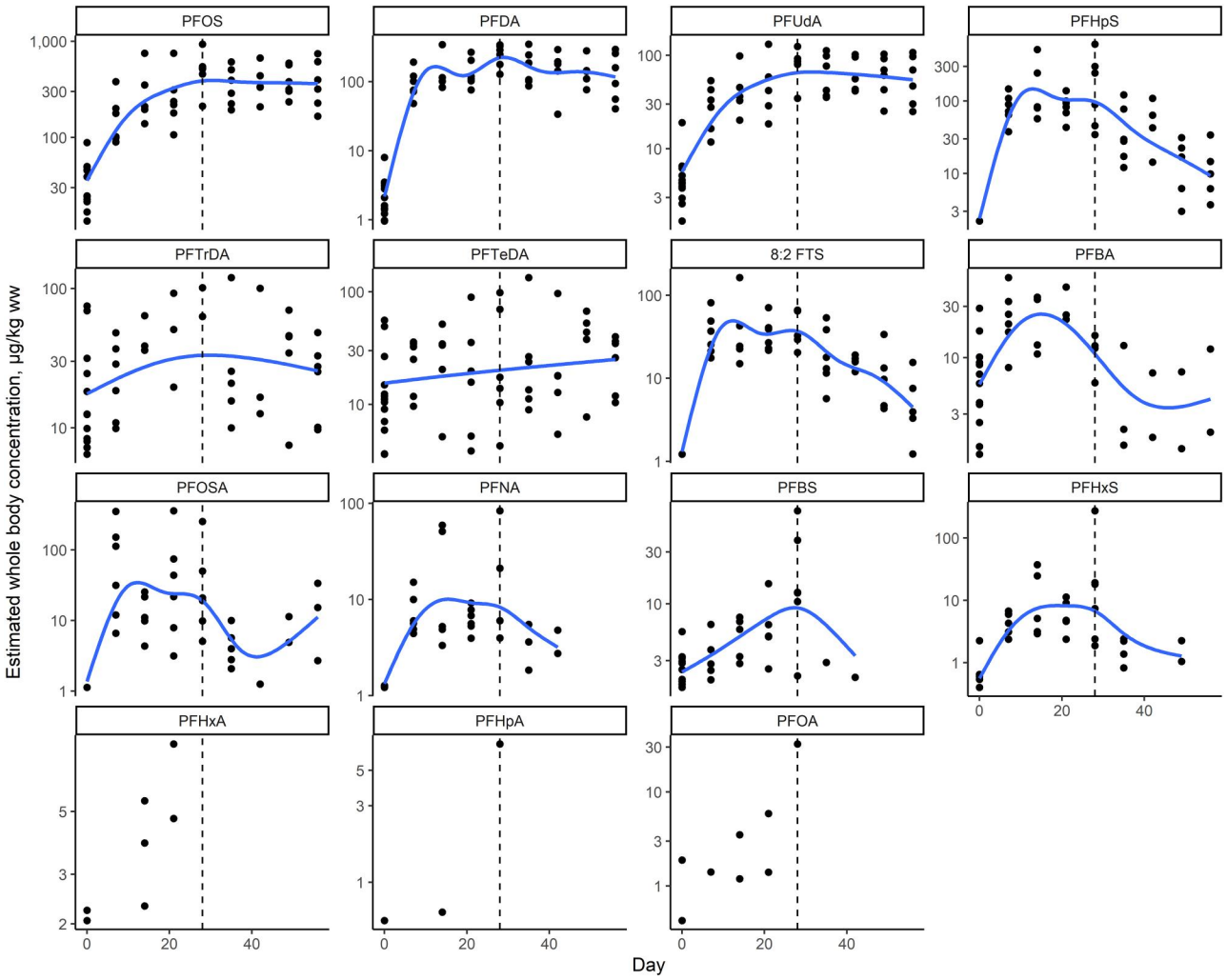

Figure 3. Estimated whole body per- and polyfluoroalkyl substance (PFAS) concentrations (µg/kg wet wt) with PFAS-wise cubic spline smoothers. Points are individuals with both liver and remainder values above the limit of quantitation, lack of points indicates incomplete data above reporting limit, and dashed vertical line indicates Day 28 transition from uptake to elimination. ww = wet weight; PFHxS = perfluorohexane sulfonic acid; PFBS = perfluorobutane sulfonic acid; PFHpS = perfluoroheptane sulfonic acid; 8:2 FTS = 8:2 fluorotelomer sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFOS = perfluorooctane sulfonic acid; PFDA = perfluorodecanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluoroocanic acid; PFUdA = perfluoroundecanoic acid; PFBA = perfluorobutanoic acid; PFHpA = perfluoroheptanoic acid; PFTeDA = perfluorotetradecanoic acid; PFTrDA = perfluorotridecanoic acid; PFHxA = perfluorohexanoic acid.

Table 1. Predicting liver concentrations of perfluorooctane sulfonate and 8:2 fluorotelomer sulfonate via the ordinary differential equation model increased mean absolute error by more than twofold[a] over the nonlinear model. Additionally, there is little influence of individual data[b] on predictive power regardless of the model choice.

		Full model	LOOCV models	Fold-change[b]
8:2 FTS	Nonlinear model MAE	15.9	16.5	1.04
	ODE model MAE	35.6	36	1.01
	Fold-change[a]	2.24	2.18	
PFOS	Nonlinear model MAE	277.2	293.5	1.06
	ODE model MAE	780.5	803.5	1.03
	Fold-change[a]	2.82	2.74	

Note. MAE = mean absolute error; LOOCV = leave-one-out cross-validation; ODE = ordinary differential equation; PFOS = perfluorooctanesulfonic acid; 8:2 FTS = 8:2 fluorotelomer sulfonic acid.
Fold-change
[a] is ODE model MAE divided by nonlinear model MAE and fold-change
[b] is LOOCV MAE divided by full model MAE.

to have TTCs that are greater than two and would be considered a likely trophic magnifier. Additionally, PFDA and perfluoroundecanoic acid (PFUdA) also have TTCs above two (Figure 4). In contrast, 8:2 FTS and PFHpS are likely to be trophic diluters or simply transfer PFAS (Figure 4). To further evaluate influence of uptake and elimination rates, the rates are presented individually in Figures 5 and 6.

Discussion

Toads were fed homogenized worms grown in a PFAS mixture-spiked soil for 28 days and then worms grown in control soil for another 28 days. Toad livers and remaining bulk tissues were collected weekly and PFAS levels quantified. Two different toxicokinetic models were fit to liver, remainder, and estimated whole body concentration data through time. A model selection procedure was used to identify a modeling approach with relatively higher predictive power. The definitive model uptake and elimination parameters were used to generate TTCs. The TTCs are intended to inform potential for trophic transfer or trophic magnification, lacking field data. The premise of single-step food web transfers informing full food web transfers is based on methods common to food web models used in ecological risk assessment

Table 2. Summary of parameter estimates from linear and nonlinear models.

PFAS	Diet, mean, (SD; μg/kg dry wt)	Tissue	Linear elimination rate (Kel) Estimate, (SE)	Nonlinear elimination rate (K_{10}) Estimate, (lower, upper)	Nonlinear uptake rate (K_{01})	Nonlinear Volume of Distribution (Vd)	Half-life (days) ln(2)/K10, (lower, upper)
PFHxS	1935.23, (354.98)	Liver	−0.068, (0.030)	−0.079, (−0.04, −0.156)	0.182, (0.049, 1)	30.301, (16.672, 35)	8.77, (17.33, 4.44)
		Remainder	−0.084, (0.045)	−0.149, (−0, −1)	0.118, (0.001, 1)	7.124, (0.34, 10)	4.65, (Inf, 0.69)
		Estimated whole body	−0.108, (0.058)	−0.154, (−0, −1)	0.139, (0.001, 1)	5.173, (0.175, 10)	4.5, (Inf, 0.69)
PFBS	1639.71, (219.4)	Liver	−0.064, (0.063)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Remainder	−0.055, (0.018)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Estimated whole body	−0.154, (0.082)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
PFHpS	1129.1, (204.72)	Liver	−0.097, (0.021)	−0.1, (−0.066, −0.147)	0.072, (0.049, 0.103)	0.32, (0.231, 0.421)	6.93, (10.5, 4.72)
		Remainder	−0.087, (0.020)	−0.097, (−0.03, −0.252)	0.063, (0.017, 0.124)	1.055, (0.431, 1.845)	7.15, (23.1, 2.75)
		Estimated whole body	−0.089, (0.019)	−0.095, (−0.038, −0.221)	0.061, (0.025, 0.117)	0.975, (0.476, 1.514)	7.3, (18.24, 3.14)
8:2 FTS	885.23, (153.27)	Liver	−0.088, (0.015)	−0.086, (−0.062, −0.115)	0.064, (0.047, 0.083)	1.31, (1.039, 1.622)	8.06, (11.18, 6.03)
		Remainder	−0.072, (0.014)	−0.074, (−0.039, −0.126)	0.081, (0.041, 0.136)	3.039, (1.914, 4.296)	9.37, (17.77, 5.5)
		Estimated whole body	−0.074, (0.013)	−0.077, (−0.043, −0.132)	0.078, (0.045, 0.134)	2.964, (1.931, 4.024)	9, (16.12, 5.25)
PFOSA	863.47, (127.96)	Liver	−0.033, (0.027)	−0.036, (−0.008, −0.069)	0.544, (0.095, 1)	2.952, (1.88, 4.353)	19.25, (86.64, 10.05)
		Remainder	−0.029, (0.032)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Estimated whole body	−0.027, (0.031)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
PFOS	697.14, (112.48)	Liver	−0.007, (0.008)	−0.009, (0.000, −0.021)	0.062, (0.028, 0.111)	0.213, (0.156, 0.266)	77.02, (Inf, 33.01)
		Remainder	−0.007, (0.009)	−0.008, (−0, −0.023)	0.073, (0.023, 0.175)	0.605, (0.404, 0.78)	86.64, (Inf, 30.14)
		Estimated whole body	−0.007, (0.008)	−0.008, (−0, −0.022)	0.065, (0.024, 0.132)	0.587, (0.393, 0.752)	86.64, (Inf, 31.51)
PFDA	629.43, (93.28)	Liver	−0.016, (0.010)	−0.017, (−0.007, −0.028)	0.065, (0.04, 0.099)	0.42, (0.336, 0.514)	40.77, (99.02, 24.76)
		Remainder	−0.023, (0.012)	−0.024, (−0.008, −0.04)	0.05, (0.024, 0.084)	0.742, (0.53, 0.99)	28.88, (86.64, 17.33)
		Estimated whole body	−0.022, (0.019)	−0.023, (−0.008, −0.039)	0.051, (0.026, 0.084)	0.755, (0.549, 0.994)	30.14, (86.64, 17.77)
PFNA	585.92, (111.88)	Liver	−0.049, (0.032)	−0.06, (−0.012, −0.123)	0.251, (0.032, 1)	5.234, (2.487, 7.954)	11.55, (57.76, 5.64)
		Remainder	−0.049, (0.038)	−0.078, (−0.014, −0.275)	0.169, (0.016, 1)	7.986, (2.416, 10)	8.89, (49.51, 2.52)
		Estimated whole body	−0.111, (0.063)	−0.214, (−0, −1)	0.064, (0.004, 0.211)	3.82, (0.345, 8.994)	3.24, (Inf, 0.69)
PFOA	276.81, (47.65)	Liver	0.043, (0.005)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Remainder	−0.158, (0.294)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Estimated whole body	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
PFUdA	192.97, (36.66)	Liver	−0.010, (0.010)	−0.012, (−0, −0.026)	0.051, (0.021, 0.092)	0.359, (0.252, 0.469)	57.76, (Inf, 26.66)
		Remainder	−0.010, (0.010)	−0.011, (−0, −0.027)	0.056, (0.019, 0.113)	0.909, (0.605, 1.215)	63.01, (Inf, 25.67)
		Estimated whole body	−0.010, (0.009)	−0.011, (−0, −0.025)	0.055, (0.023, 0.104)	0.899, (0.628, 1.177)	63.01, (Inf, 27.73)

(continued)

Table 2. (continued)

PFAS	Diet, mean, (SD; μg/kg dry wt)	Tissue	Linear elimination rate (Kel) Estimate, (SE)	Nonlinear elimination rate (K_{10}) Estimate, (lower, upper)	Nonlinear uptake rate (K_{01})	Nonlinear Volume of Distribution (Vd)	Half-life (days) ln(2)/K10, (lower, upper)
PFBA	160.03, (354.4)	Liver	–0.024, (0.028)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Remainder	–0.024, (0.019)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Estimated whole body	–0.031, (0.025)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
PFHpA	90.69, (15.67)	Liver	0.005, (0.023)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Remainder	–0.173, (0.192)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Estimated whole body	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
PFTeDA	36.96, (4.96)	Liver	0.010, (0.017)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Remainder	0.011, (0.017)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Estimated whole body	0.011, (0.017)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
PFTrDA	13.31, (2.08)	Liver	–0.010, (0.018)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Remainder	–0.010, (0.019)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Estimated whole body	–0.023, (0.019)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
PFHxA	1.86, (0.33)	Liver	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Remainder	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)
		Estimated whole body	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)	NA, (NA)

Note. NA indicates insufficient data or poorly performing model fit (e.g., concentrations are indistinguishable from background). toad data are wet weight, rates are unitless multiplicative rates but should be considered as fractions of wet weight. Upper = 97.5th percentile; lower = 2.5th percentile; PFHxS = perfluorohexane sulfonic acid; PFBS = perfluorobutane sulfonic acid; PFHpS = perfluoroheptane sulfonic acid; 8:2 FTS = 8:2 fluorotelomer sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFOS = perfluorooctane sulfonic acid; PFDA = perfluorodecanoic acid; PFNA = perfluononanoic acid; PFOA = perfluoroocanic acid; PFUdA = perfluoroundecanoic acid; PFBA = perfluorobutanoic acid; PFHpA = perfluoroheptanoic acid; PFTeDA = perfluorotetradecanoic acid; PFTrDA = perfluorotridecanoic acid; PFHxA = perfluorohexanoic acid.

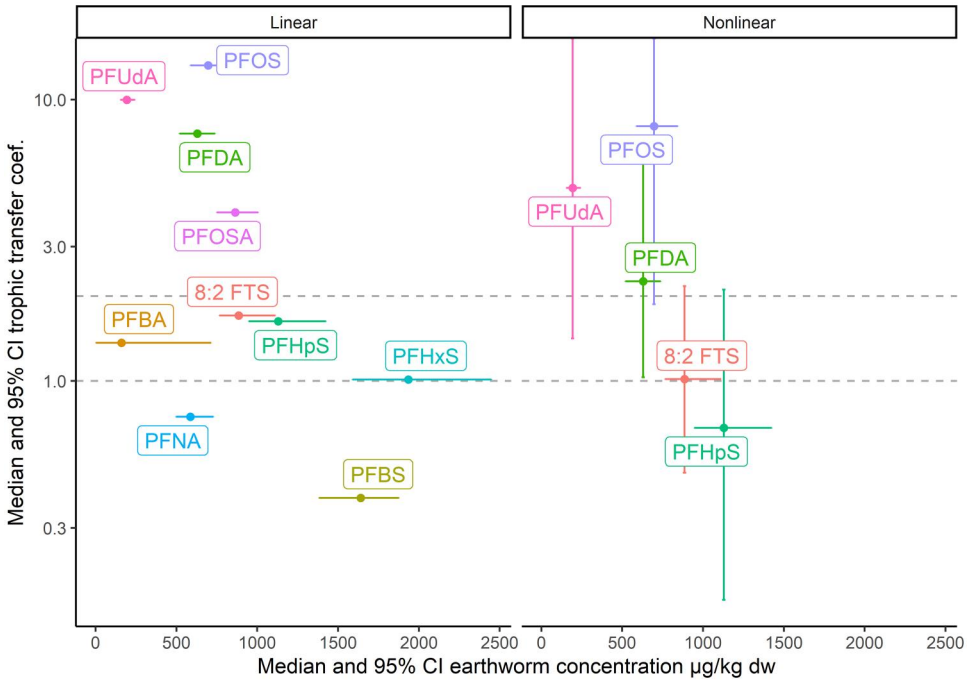

Figure 4. Trophic transfer coefficients (TTCs) in relation to diet concentration (µg/kg dry wt) for definitive nonlinear model (right) and informative but "pilot" linear models (left). Points are estimates and lines are upper and lower 95% confidence intervals from diet data (horizontal) or resampled bootstrap parameter distributions (vertical). Dashed horizontal lines are at 1.0 and 2.0 and indicate a transition area where per- and polyfluoroalkyl substances are more likely to be trophic magnifiers ($y > 2$), trophic diluters ($y < 1$), or simply transfer ($1 < y < 2$). dw = dry weight; PFHxS = perfluorohexane sulfonic acid; PFBS = perfluorobutane sulfonic acid; PFHpS = perfluoroheptane sulfonic acid; 8:2 FTS = 8:2 fluorotelomer sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFOS = perfluorooctane sulfonic acid; PFDA = perfluorodecanoic acid; PFNA = perfluorononanoic acid; PFUdA = perfluoroundecanoic acid; PFBA = perfluorobutanoic acid.

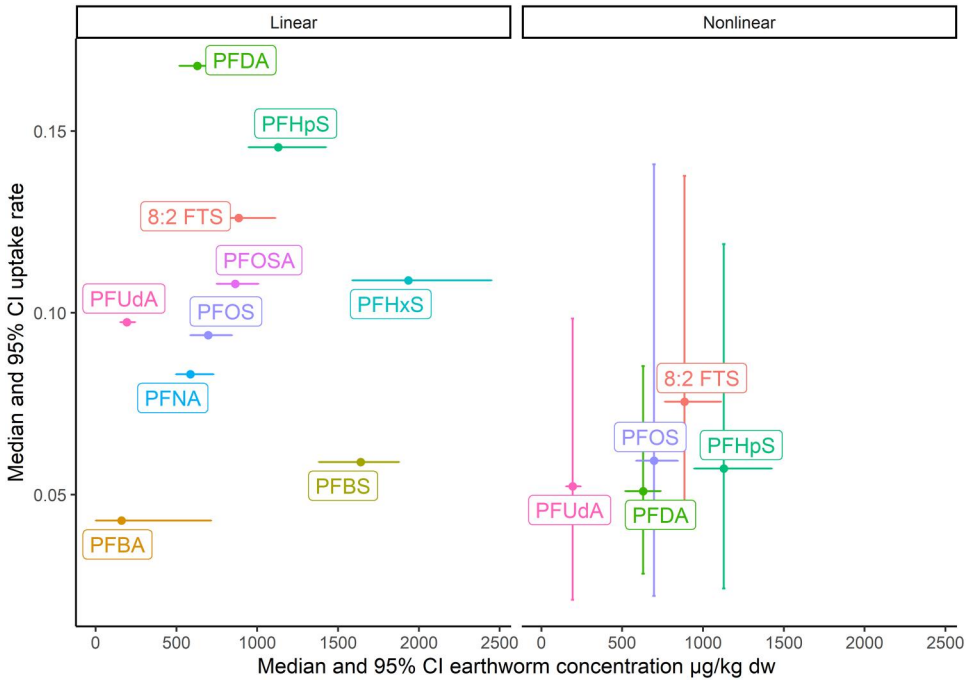

Figure 5. Uptake rates via definitive nonlinear models (right) and pilot linear models (left) in relation to diet concentration (µg/kg dry wt). Note that accounting for volume of distribution (Vd) lowers the uptake rate and indicates that uptake rates across per- and polyfluoroalkyl substances are similar, but other parameters vary. Points are means, error bars are 95% confidence intervals. Horizontal errors bars based on sample size of five, vertical error bars based on resampled bootstrap parameter estimates of uptake rate. dw = dry weight; CI = confidence interval; PFHxS = perfluorohexane sulfonic acid; PFBS = perfluorobutane sulfonic acid; PFHpS = perfluoroheptane sulfonic acid; 8:2 FTS = 8:2 fluorotelomer sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFOS = perfluorooctane sulfonic acid; PFDA = perfluorodecanoic acid; PFNA = perfluorononanoic acid; PFUdA = perfluoroundecanoic acid; PFBA = perfluorobutanoic acid.

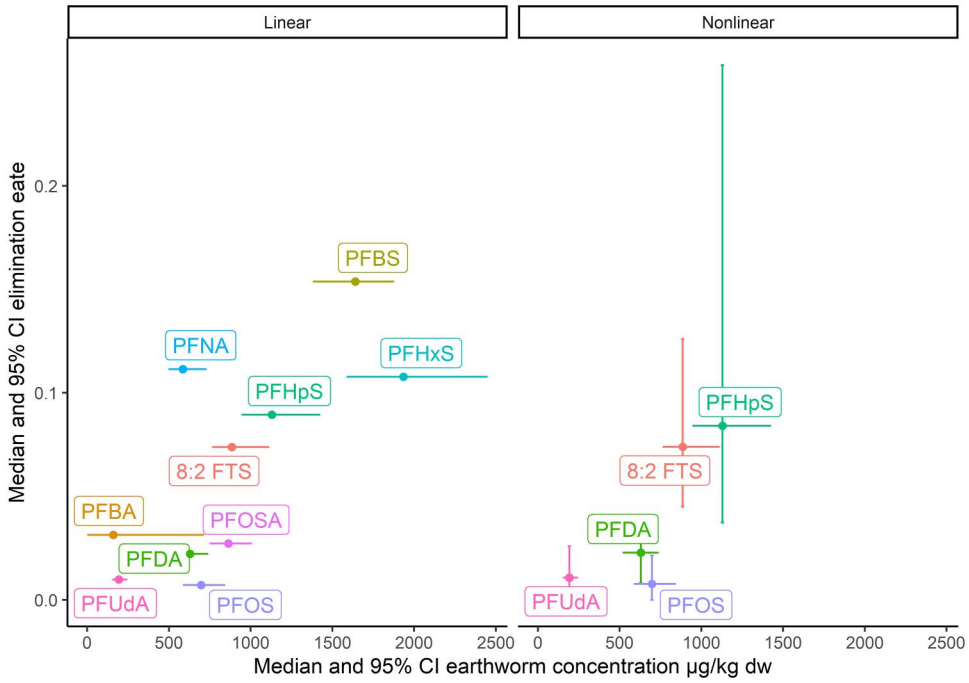

Figure 6. Elimination rates for definitive nonlinear models (right) and pilot linear models (left) in relation to diet concentration (µg/kg dry wt). Points are means, error bars are 95% confidence intervals. Horizontal errors bars based on sample size of five, vertical error bars based on resampled bootstrap parameter estimates of uptake rate. dw = dry weight; CI = confidence interval; PFHxS = perfluorohexane sulfonic acid; PFBS = perfluorobutane sulfonic acid; PFHpS = perfluoroheptane sulfonic acid; 8:2 FTS = 8:2 fluorotelomer sulfonic acid; PFOSA = perfluorooctane sulfonamide; PFOS = perfluorooctane sulfonic acid; PFDA = perfluorodecanoic acid; PFNA = perfluorononanoic acid; PFUdA = perfluoroundecanoic acid; PFBA = perfluorobutanoic acid.

(e.g., Larson et al., 2018) and the definitions of bioaccumulation and biomagnification factors (Burkhard et al., 2012; Gobas et al., 2016).

The TTCs for PFOS, PFUdA, and PFDA are all above two and indicate that trophic magnification is possible in this kinetics-based screening approach. Note that lower confidence levels in all three of these PFAS fall below two, but for PFOS and PFUdA, the upper confidence limits are infinity. This is due to elimination rates for PFOS and PFUdA including 0 (see Table 2 rates and half-lives). In contrast, the PFAS that are expected to be trophic diluters (8:2 FTS and PFHpS) have nearly equivalent elimination and uptake rates. They also have widely varying TTCs, which can likely be attributed to generating ratios from lower variability numerators and denominators; a 5%–10% multiplicative uptake or elimination rate variation is less alarming than a many-fold TTC variation. Although there are highly variable patterns in TTCs, we note the potential for dilution is less strong than magnification; TTC central estimates for 8:2 FTS and PFHpS are between 1–0.3, whereas PFOS and PFUdA are between 3–10. Across PFAS, uptake rates are somewhat clustered between 0.05 and 0.1 (Figure 5) and elimination rates are more variable, ranging from 0.01–0.1 (Figure 6). Further, elimination rates are somewhat clustered with PFOS, PFUdA, and PFDA below 0.05 and PFHpS and 8:2 FTS between 0.05–0.1. Accordingly, a plausible broad hypothesis is that TTC values are a function of elimination rate. Our approach and interpretation of these individual-level rates as informative of larger system processes (trophic transfer) is essentially a simplification of the individual toxicokinetic models used in aquatic organismal models used to predict bioconcentration, bioaccumulation, biomagnification, and trophic transfer (Kelly et al., 2024; Mackay et al., 2016, 2018; Sun et al., 2022).

The relationship between diet concentration and TTC, uptake, or elimination rates are unclear (Figure 4). Because the linear model for elimination rates is less problematic than linear uptake rates, the elimination rates are reasonably comparable to the nonlinear parameter estimates. Figure 6 demonstrates a potential relationship between elimination rate and diet; as concentrations rise, elimination tends to increase. Given that uptake is expected to be consistent across PFAS, it is plausible to hypothesize that TTCs are driven by elimination, and elimination may be driven by a concentration dependent process. Although elimination rates are not provided in Burkhard and Votava (2022), if earthworm biota-sediment accumulation factors (BSAF) values decrease with increasing concentrations, and we assume that BSAF is a function of underlying uptake and elimination processes, an increase in elimination rates could be explanatory in the earthworm observations. Lin et al. (2023) identified that organic anion transport mechanisms are saturable and likely explain the variable PFOA half-life lengths observed in humans. However, their observation would suggest that at high concentrations, elimination is decreased. Resolution of concentration dependent mechanisms remains unclear, but the importance of elimination is clear.

Half-lives appear to be in several clusters with some central estimates at less than 28 days (e.g., 8:2 FTS), some very near 28 days (e.g., PFDA), and several much longer than 28 days (e.g., PFOS). These estimates (and their related parameter estimates) are unclearly related to diet concentration. Similarly, although there does appear to be a pattern of the increasing half-life with fluorinated carbon chain length, there is clearly a relationship with functional group, because PFNA, PFOS, and 8:2 FTS have similar fluorinated carbon chain lengths but capture the lowest, average, and highest half-lives (see Table 2). It should also be noted that these half-lives are based on the elimination rates determined in the nonlinear model effort and accordingly account for kinetics. Our expectation is that this representation of half-

life is more inclusive of the multiple processes (uptake, volume of distribution, and elimination) that influence PFAS internal dose. See discussions of individual studies below, but few data exist that could inform our PFAS-specific observations.

Another critical observation of this study is that PFAS that tend to accumulate in worms may not necessarily accumulate in toads. Perfluorohexane sulfonate, as the highest concentration PFAS observed in worms, had such large elimination rates in toads that model parameter estimates were challenging (95% confidence intervals span 0–1; see also Lotufo et al., 2025). Although there is concern about concentration-dependent elimination rate, PFAS, such as PFOS, may have a medium categorized BAF (ratio of worm tissue concentration to soil concentration is a central observation; Lotufo et al., 2025) but a high categorized TTC (the highest TTC; in this study, at the toad trophic level). Contrasting, 8:2 FTS has a medium categorized BAF but a low categorized TTC. So, although concentration-dependent elimination may be observed, these data indicate that the PFAS and its kinetics are the drivers of trophic transfer (Kelly et al., 2024). This is in alignment with the actual scientific question in mind—how do we better describe the PFAS–organism relationship in the laboratory such that we can better understand field observations?

Notable field observations from terrestrial systems generally do not include herptile taxa. Those studies that do include herptile taxa are often in single trophic steps and do not meet definitions of food web studies where trophic transfer would be quantified. However, for comparison of the PFAS-to-PFAS patterns, we discuss some studies. In Müller et al. (2011), the food chain described was lichen to reindeer to wolves, in Huang et al. (2022), grass, pika, and eagle were sampled, and in Fremlin et al. (2023), invertebrates, songbirds, and hawk eggs were sampled. Heimstad et al. (2024) sampled invertebrates, songbird eggs, and hawk eggs (with other mammalian tissues in a nonlinear food web) Thus, although these studies do not provide a direct comparison of an invertebrate to toad, PFAS-specific comparisons may be useful. Perfluorooctane sulfonate, in particular, shows the most potential for trophic magnification in these field data. This is in alignment with the PFOS data from this study. This is also in alignment with short food web studies that tracked PFOS alone from soil through worms and plants to mice and identified BMFs greater than 1 for PFOS (D'Hollander et al., 2014) or multiple PFAS from soil through worms to voles and identified BMFs greater than 1 for PFOS alone (all other PFAS were < 1; Grønnestad et al., 2019). Also note that long chain perfluoroalkyl carboxylates PFDA and PFUdA are high potential magnifiers in this study, but that principle may not be consistently observed in the field sites. In Müller et al. (2011), the larger PFAS have a reduction in trophic magnification factors, but in Huang et al. (2022) and Fremlin et al. (2023), these PFAS may show trophic magnification levels that are near to PFOS. In Heimstad et al. (2024), the long chain perfluorocarboxylates show an increase and then decreasing pattern with a peak at perfluorododecanoic acid. Note that perfluorohexadecanoic acid was indistinguishable from zero (Heimstad et al., 2024). Cui et al. (2018) evaluated adult Ranid frogs (*Pelophylax nigromaculatus*) living in wet agricultural habitats. Their bioconcentration factor values (frog tissue concentration/water concentration; unclearly described as BAFs, implying dietary exposure) increase with carbon chain length for perfluoroalkyl carboxylates and sulfonates and their chlorinated replacements (Cui et al., 2018). It remains untested how our individual trophic transition (diet to toad) could speak to larger food chains with multiple trophic transfers, but some alignment suggests that overall trophic transfer of PFAS may be a function of internal toxicokinetic rates; if bioaccumulation is informative of biomagnification, we should observe alignment of individual toxicokinetic rates and system level trophic magnification rates.

Herptile laboratory data largely exist for their aquatic, larval stages. The data are lower resolution across time but do allow for a comparative basis. For instance, Ankley et al. (2004) provide bioconcentration information in larval ranids (*Lithobates pipiens*, formerly *Rana pipiens*) that were observed during a toxicity test. Values indicate bioconcentration factors based on ratios of estimated tissue concentrations and water concentrations to be lower than those bioconcentration factors based on ratio of kinetic parameters (uptake vs. elimination). Importantly, given the range of concentrations in water and tissues, bioconcentration values ranged from 17.5–175 (Ankley et al., 2004). Assuming that our dietary TTCs represent one exposure route and magnitude at a terrestrial life stage and the bioconcentration another exposure route and magnitude in an aquatic life stage, across the full life of any given amphibian, the bioconcentration factor may be higher than dietary accumulation. The implication is that PFAS burdens from aquatic systems are, at the least, equivalently important as dietary exposure during adulthood. However, the continuity of body burdens across life stages assumes continuous uptake and/or low elimination. Disregarding the difference between *Lithobates* (Ranid frog) and *Ambystoma* (Ambystomatid salamander), the data from Flynn et al. (2021) would concur that for PFOS, a biomagnification factor based on diet to salamander tissue between 1–3 would be lower than bioaccumulation in larval *Lithobates*. Interestingly, as dermal exposure is highly relevant for taxa that have generally permeable skin (amphibians), data on dermal transfer indicates that PFAS may or may not have influential BSAFs. Abercrombie et al. (2021) indicate that in *Ambystoma* PFOA, PFOS, PFHxS, and 6:2 FTS have BSAF values that are at or below 0.10, indicating that dermal routes are not routes of high accumulation. Note, however, that these values are similar to the dietary accumulation factors of Flynn et al. (2021) for PFOA, PFHxS, and 6:2 FTS. Accordingly, the more insightful observation is that dietary accumulation is only higher for PFOS. Further, Abercrombie et al. (2021) include *Anaxyrus americanus* data, so a comparison can be directly made to our data. The BSAF values for dermal exposure to PFOA, PFHxS, and 6:2 FTS are all below 0.1 and for PFOS range between 0.1–0.25 (Abercrombie et al., 2021). This indicates that the dietary exposure route is more accumulative than dermal for PFOS, but low TTCs for PFOA, PFHxS indicate that dermal accumulation may be comparable to dietary accumulation. The relationship between 8:2 FTS and 6:2 FTS remains untested in these taxa. Our data, in conjunction with some other amphibian data (Abercrombie et al., 2021; Flynn et al., 2021), suggest that PFOS is one PFAS that has high dietary accumulation during terrestrial life stages and low enough elimination in aquatic and terrestrial life stages to persist in an individual through an aquatic to terrestrial transition.

Data related to toxicity of PFOS, PFOA, PFHxS, chlorinated polyfluorinated ether sulfonic acid (6:2 Cl-PFESA), and hexafluoropropylene oxide trimer acid (HFPO-TA) in terrestrial life stage amphibians is only available in dermal exposure routes (Abercrombie et al., 2021; Lin et al., 2022) and via diet in salamanders (Flynn et al., 2021). Other PFAS studied here do not have data available. However, exposure via dermal routes to PFOS, PFOA, and PFHxS did not reveal detectable dose-response data effects up to 8,000 ppb dry weight basis (Abercrombie et al., 2021). Yet, comparison back to control data suggests that potentially a no-effect concentration was unobserved, and actually, all

treatments were indicative of toxic effects. Although there is limited amphibian data, at the least, PFOS and PFHxS appear to be more toxic than PFOA (6:2 FTS may be the most impactful; Abercrombie et al., 2021). Flynn et al. (2021) indicate that PFOS significantly reduces growth using a marginal approach to control for mismatched starting weights. As summarized in Pandelides et al. (2023), this effect measure may not be one risk assessors are comfortable with. Regardless, the rejection of the relevance of these impacts (by Pandelides et al. [2023]) is not compatible with modern statistical thinking nor is "weight at end of study" compatible with population-relevant models that would rely on growth rate (i.e., a marginal measure). Lin et al. (2022) identified increased hepatosomatic index and mechanistic signals of lipid dysregulation in adult ranid frogs (*Pelophylax nigromaculatus*) exposed to 1–10 μg/L PFOA, PFOS, 6:2 Cl-PFESA, and HFPO-TA. Importantly, the interpretation was that replacement PFAS were equally toxic as legacy PFAS (Lin et al., 2022). Uncertainty about comparability of strictly aquatic exposure versus dietary should be noted here as well as the general lack of utility for suborganismal data in risk assessments (Pandelides et al., 2023).

Exposure through contaminated bedding is not addressed here but is addressed by Abercrombie et al. (2021). However, it is likely important in real field settings, as toads absorb moisture through their stomach skin/ventral surfaces. The toads in this study were observed resting in their Petri dish water supplies regularly. The water provided is based on source water that is PFAS-free (Narizzano et al., 2024), but it is not determined whether elimination and or uptake could be occurring through this dermal route in these organisms. Abercrombie et al. (2021) would likely suggest dermal exposure to any contamination is low, but this cannot be addressed, as this study was not designed to answer these questions. In contrast, Cui et al. (2018) indicate that substantial portions of the PFAS burden in frogs may be in the skin tissue itself. Although one could hypothesize that smaller and/or more water soluble PFAS would tend to move through the porous skin of amphibians, potentially the relatively lower flux observed by Abercrombie et al. (2021) can be explained by deposition into the skin. Elimination through feces or molting skin may increase variability in observed concentrations as fecal deposition is periodic (daily to several days between events) and molting of skin may occur sporadically and requires high quality laboratory care to occur in the laboratory.

Uncertainties in this dataset and analysis are largely related to corroboration with field data with multiple trophic levels with amphibians. There are also few laboratory data with terrestrial life stage amphibians, but further, there are limited PFAS trophic transfer studies in the laboratory. McDermett et al. (2022) and Judy et al. (2022) describe the movement of PFAS from plants to invertebrates, so there is not a direct relation to this study, as the invertebrates in our study were exposed directly to the soil, not via diet alone. Importantly, the McDermett et al. (2022) and Judy et al. (2022) studies suggest (1) trophic dilution through this food chain and (2) PFOS is the highest trophic "magnification" factor. The worm, soil, PFAS combination described here (Kuperman et al., 2025) suggests that some PFAS are diluters in the soil-worm step (shorter chain PFAS), but others are accumulative (longer chain PFAS) and would suggest trophic magnification. The resultant hypothesis that could be explored to address this uncertainty is that water-plant transfer is where dilution emerges whereas soil+water-invert-predator is a magnifying pathway. Although there are features of PFAS congeners that are influential, the features of the organism or food chain of interest and the abiotic media source are likely equally influential. As an example, Scearce et al. (2023) indicate that characteristics of the PFAS, soil, ground/surface water, and the plants are all interacting in a manner that has left predicting uptake into sessile organisms (plants) a challenge.

Another uncertainty that cannot be resolved in this study design is the nonlinear trophic magnification factors~CF-chain length patterns observed in the field (Fremlin et al., 2023; Huang et al., 2022; Müller et al., 2011) against the relatively linear BAF~CF-chain length patterns observed in single step transfers (Burkhard & Votava, 2022). Importantly, however, it can easily be hypothesized that in the field, higher level organisms' diets, even with a relatively well defined/constrained food chain, are an integration of a variety of physicochemical and biological processes. So, although a linear pattern may exist in a basal food chain step (soil to worm), at higher trophic levels, the CF-chain length may not be entirely explanatory; see the mismatch in adult ranid frog (*Pelophylax nigromaculatus*) field versus laboratory frog tissue concentration/water concentration in Lin et al. (2022), where field bioconcentration is substantially higher than observations in the laboratory.

In conclusion, we utilized internal toxicokinetics of dietary exposure to a PFAS mixture in a terrestrial life stage amphibian to identify PFAS trophic transfer coefficients. The PFAS with relatively high TTCs are suggested to likely be trophic magnifiers (PFOS, PFUdA, and PFDA), 8:2 FTS is likely to equivalently transfer, and PFHpS a trophic diluter. These data are somewhat as expected from some field data (Fremlin et al., 2023; Huang et al., 2022; Müller et al., 2011) where PFOS is expected to by a trophic magnifier. The novel addition from this study is that in higher order organisms, this observation may be a function of very low elimination at the individual level. At the primary consumers, the trophic magnification of PFOS may be considered medium, in that our BAFs were in the middle of the span of BAFs observed (Kuperman et al., 2025). In concert, the field observations may be a function of uncaptured processes, specifically for PFOS.

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

Author contributions

Andrew G. East (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing—original draft), Michael Siimini (Data curation, Investigation, Methodology, Writing—review & editing), Emily E. Stricklin (Data curation, Investigation, Methodology, Writing—review & editing), Guilherme R. Lotufo (Data curation, Investigation, Methodology, Writing—review & editing), Jennifer Guelfo (Data curation, Investigation, Methodology, Writing—review & editing), Zhao Yang (Data curation, Investigation, Methodology, Writing—review & editing), Travis Gallo (Methodology, Software, Writing—review & editing), Michael J. Quinn (Investigation, Writing—review & editing), and Roman G. Kuperman (Conceptualization, Funding acquisition, Methodology, Supervision, Writing—review & editing)

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Conflicts of interest

The authors declare no conflicts of interest.

Disclaimer

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Ethics statement

Animal use was approved by DCPH-A Animal Care and Use Committee (animal use protocol number: 06-22-02-02).

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References

Abercrombie, S. A., de Perre, C., Iacchetta, M., Flynn, R. W., Sepúlveda, M. S., Lee, L. S., & Hoverman, J. T. (2021). Sublethal effects of dermal exposure to poly- and perfluoroalkyl substances on postmetamorphic amphibians. *Environmental Toxicology and Chemistry*, *40*, 717–726. https://doi.org/10.1002/etc.4711

Anderson, D. R. (2008). *Model based inference in the life sciences: A primer on evidence*. Springer New York. http://link.springer.com/10.1007/978-0-387-74075-1

Ankley, G. T., Kuehl, D. W., Kahl, M. D., Jensen, K. M., Butterworth, B. C., & Nichols, J. W. (2004). Partial life-cycle toxicity and bioconcentration modeling of perfluorooctanesulfonate in the northern leopard frog (*Rana pipiens*). *Environmental Toxicology and Chemistry*, *23*, 2745–2755. https://doi.org/10.1897/03-667

Baty, F., Ritz, C., Charles, S., Brutsche, M., Flandrois, J.-P., & Delignette-Muller, M.-L. (2015). A Toolbox for nonlinear regression in *R* : The package nlstools. *Journal of Statistical Software*, *66*, 1–21. https://doi.org/10.18637/jss.v066.i05

Burkhard, L. P., & Votava, L. K. (2022). Review of per- and polyfluoroalkyl substances (PFAS) bioaccumulation in earthworms. *Environmental Advances*, *11*, 1. https://doi.org/10.1016/j.envadv.2022.100335

Burkhard, L. P., Arnot, J. A., Embry, M. R., Farley, K. J., Hoke, R. A., Kitano, M., Leslie, H. A., Lotufo, G. R., Parkerton, T. F., Sappington, K. G., Tomy, G. T., & Woodburn, K. B. (2012). Comparing laboratory and field measured bioaccumulation endpoints. *Integrated Environmental Assessment and Management*, *8*, 17–31. https://doi.org/10.1002/ieam.260

Choi, Y. J., Lee, L. S., Hoskins, T. D., Gharehveran, M. M., & Sepúlveda, M. S. (2023). Occurrence and implications of per and polyfluoroalkyl substances in animal feeds used in laboratory toxicity testing. *Science of The Total Environment*, *867*, 161583. https://doi.org/10.1016/j.scitotenv.2023.161583

Cui, Q., Pan, Y., Zhang, H., Sheng, N., Wang, J., Guo, Y., & Dai, J. (2018). Occurrence and tissue distribution of novel perfluoroether carboxylic and sulfonic acids and legacy per/polyfluoroalkyl substances in black-spotted frog (*Pelophylax nigromaculatus*). *Environmental Science & Technology*, *52*, 982–990. https://doi.org/10.1021/acs.est.7b03662

D'Hollander, W., De Bruyn, L., Hagenaars, A., De Voogt, P., & Bervoets, L. (2014). Characterisation of perfluorooctane sulfonate (PFOS) in a terrestrial ecosystem near a fluorochemical plant in Flanders, Belgium. *Environmental Science and Pollution Research*, *21*, 11856–11866. https://doi.org/10.1007/s11356-013-2449-4

East, A. G., Anderson, R. H., Duncan, C. M., & Salice, C. J. (2025). Surface soil per- and polyfluoroalkyl substance mixtures dominated by perfluorooctane sulfonate: Prioritization for ecotoxicity testing and ecological risk assessment at current and former U.S. Air Force bases. *Environmental Toxicology and Chemistry*, *44*, 856–865. https://doi.org/10.1093/etojnl/vgaf001

Elzhov, T. V., Mullen, K. M., Spiess, A.-N., & Bolker, B. (2023). minpack.lm: R Interface to the Levenberg-Marquardt Nonlinear Least-Squares Algorithm Found in MINPACK, Plus Support for Bounds. https://CRAN.R-project.org/package=minpack.lm.

Evich, M. G., Davis, M. J. B., McCord, J. P., Acrey, B., Awkerman, J. A., Knappe, D. R. U., Lindstrom, A. B., Speth, T. F., Tebes-Stevens, C., Strynar, M. J., Wang, Z., Weber, E. J., Henderson, W. M., & Washington, J. W. (2022). Per- and polyfluoroalkyl substances in the environment. *Science*, *375*, eabg9065. https://doi.org/10.1126/science.abg9065

Finkler, M. S., Hayes, C. J., & Rifai, L. (2014). Sexual dimorphisms in metabolism, organ mass, and reproductive energetics in pre-breeding American toads (*Anaxyrus americanus*). *Copeia*, *2014*, 447–453. https://doi.org/10.1643/CP-13-167

Flynn, R. W., Hoskins, T. D., Iacchetta, M., De Perre, C., Lee, L. S., Hoverman, J. T., & Sepulveda, M. S. (2021). Dietary exposure and accumulation of per- and polyfluoroalkyl substances alters growth and reduces body condition of post-metamorphic salamanders. *Science of the Total Environment*, *765*, 142730. https://doi.org/10.1016/j.scitotenv.2020.142730

Fremlin, K. M., Elliott, J. E., Letcher, R. J., Harner, T., & Gobas, F. A. P. C. (2023). Developing methods for assessing trophic magnification of perfluoroalkyl substances within an urban terrestrial avian food web. *Environmental Science & Technology*, *57*, 12806–12818. https://doi.org/10.1021/acs.est.3c02361

Giesy, J. P., & Kannan, K. (2001). Global distribution of perfluorooctane sulfonate in wildlife. *Environmental Science & Technology*, *35*, 1339–1342. https://doi.org/10.1021/es001834k

Gobas, F. A., Burkhard, L. P., Doucette, W. J., Sappington, K. G., Verbruggen, E. M., Hope, B. K., Bonnell, M. A., Arnot, J. A., & Tarazona, J. V. (2016). Review of existing terrestrial bioaccumulation models and terrestrial bioaccumulation modeling needs for organic chemicals. *Integrated Environmental Assessment and Management*, *12*, 123–134. https://doi.org/10.1002/ieam.1690

Grønnestad, R., Vázquez, B. P., Arukwe, A., Jaspers, V. L. B., Jenssen, B. M., Karimi, M., Lyche, J. L., & Krøkje, Å. (2019). Levels, patterns, and biomagnification potential of perfluoroalkyl substances in a terrestrial food chain in a Nordic skiing area. *Environmental Science & Technology*, *53*, 13390–13397. https://doi.org/10.1021/acs.est.9b02533

Heimstad, E. S., Nygård, T., Moe, B., & Herzke, D. (2024). New insights from an eight-year study on per- and polyfluoroalkyl substances in an urban terrestrial ecosystem. *Environmental Pollution*, *347*, 123735. https://doi.org/10.1016/j.envpol.2024.123735

Houde, M., Czub, G., Small, J. M., Backus, S., Wang, X., Alaee, M., & Muir, D. C. G. (2008). Fractionation and bioaccumulation of perfluorooctane sulfonate (PFOS) isomers in a Lake Ontario food

web. *Environmental Science & Technology*, *42*, 9397–9403. https://doi.org/10.1021/es800906r

Huang, K., Li, Y., Bu, D., Fu, J., Wang, M., Zhou, W., Gu, L., Fu, Y., Cong, Z., Hu, B., Fu, J., Zhang, A., & Jiang, G. (2022). Trophic magnification of short-chain per- and polyfluoroalkyl substances in a terrestrial food chain from the Tibetan Plateau. *Environmental Science and Technology Letters*, *9*, 147–152. https://doi.org/10.1021/acs.estlett.1c01009

Judy, J. D., Gravesen, C., Christopher Wilson, P., Lee, L., Sarchapone, J., Hinz, F., & Broadbent, E. (2022). Trophic transfer of PFAS from tomato (*Solanum lycopersicum*) to tobacco hornworm (*Manduca sexta*) caterpillars. *Environmental Pollution*, *310*, 119814. https://doi.org/10.1016/j.envpol.2022.119814

Kelly, B. C., Sun, J. M., McDougall, M. R. R., Sunderland, E. M., & Gobas, F. A. P. C. (2024). Development and evaluation of aquatic and terrestrial food web bioaccumulation models for per- and polyfluoroalkyl substances. *Environmental Science & Technology*, *58*, 17828–17837. https://doi.org/10.1021/acs.est.4c02134

Kuperman, R. G., Simini, M., Wright, L. K., Moretz, R., Stricklin, E. E., Lotufo, G. R., Boyd, R., East, A. G., Quinn, M. J., Guelfo, J., & Yang, Z. (2025). Determination of biomagnification potentials for per- and polyfluoroalkyl substances in terrestrial food webs: final report. U.S. Army Combat Capabilities Development Command Chemical Biological Center Report No.: DEVCOM CBC-TR-1919.

Larson, E. S., Conder, J. M., & Arblaster, J. A. (2018). Modeling avian exposures to perfluoroalkyl substances in aquatic habitats impacted by historical aqueous film forming foam releases. *Chemosphere*, *201*, 335–341. https://doi.org/10.1016/j.chemosphere.2018.03.004

Lin, H., Liu, Z., Yang, H., Lu, L., Chen, R., Zhang, X., Zhong, Y., & Zhang, H. (2022). Per- and Polyfluoroalkyl Substances (PFASs) impair lipid metabolism in *Rana nigromaculata* : A field investigation and laboratory study. *Environmental Science & Technology*, *56*, 13222–13232. https://doi.org/10.1021/acs.est.2c03452

Lin, J., Chin, S. Y., Tan, S. P. F., Koh, H. C., Cheong, E. J. Y., Chan, E. C. Y., & Chan, J. C. Y. (2023). Mechanistic middle-out physiologically based toxicokinetic modeling of transporter-dependent disposition of perfluorooctanoic acid in humans. *Environmental Science & Technology*, *57*, 6825–6834. https://doi.org/10.1021/acs.est.2c05642

Lotufo, G., Boyd, R. E., Krupa, P. M., Yang, Z., Guelfo, J. E., Simini, M., & Kuperman, R. G. (2025). Uptake and elimination of per- and polyfluoroalkyl substances in earthworms exposed to spiked artificial soil or aqueous medium or artificial soil. *Environmental Toxicology and Chemistry*, *44*, 1655–1671. https://doi.org/10.1093/etojnl/vgaf083

Mackay, D., Celsie, A. K. D., Arnot, J. A., & Powell, D. E. (2016). Processes influencing chemical biomagnification and trophic magnification factors in aquatic ecosystems: Implications for chemical hazard and risk assessment. *Chemosphere*, *154*, 99–108. https://doi.org/10.1016/j.chemosphere.2016.03.048

Mackay, D., Celsie, A. K. D., Powell, D. E., & Parnis, J. M. (2018). Bioconcentration, bioaccumulation, biomagnification and trophic magnification: a modelling perspective. *Environmental Science: Processes and Impacts*, *20*, 72–85. https://doi.org/10.1039/C7EM00485K

Malinsky, M. D., Jacoby, C. B., & Reagen, W. K. (2011). Determination of perfluorinated compounds in fish fillet homogenates: Method validation and application to fillet homogenates from the Mississippi River. *Analytica Chimica Acta*, *683*, 248–257. https://doi.org/10.1016/j.aca.2010.10.028

Martin, J. W., Mabury, S. A., Solomon, K. R., & Muir, D. C. G. (2003a). Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry*, *22*, 189–195. https://doi.org/10.1002/etc.5620220125

Martin, J. W., Mabury, S. A., Solomon, K. R., & Muir, D. C. G. (2003b). Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry*, *22*, 196–204. https://doi.org/10.1002/etc.5620220126

McDermett, K., Anderson, T., Jackson, W. A., & Guelfo, J. (2022). Assessing potential perfluoroalkyl substances trophic transfer to crickets (*Acheta domesticus*). *Environmental Toxicology and Chemistry*, *41*, 2981–2992. https://doi.org/10.1002/etc.5478

Müller, C. E., De Silva, A. O., Small, J., Williamson, M., Wang, X., Morris, A., Katz, S., Gamberg, M., & Muir, D. C. G. (2011). Biomagnification of perfluorinated compounds in a remote terrestrial food chain: Lichen–caribou–wolf. *Environmental Science & Technology*, *45*, 8665–8673. https://doi.org/10.1021/es201353v

Narizzano, A. M., Lent, E. M., East, A. G., Bohannon, M. E., & Quinn, M. J. (2024). Threshold for increased liver weight is protective of other effects in *Peromyscus* exposed to PFNA. *Toxicological Sciences*, *201*, 38–47. https://doi.org/10.1093/toxsci/kfae077

National Research Council (U.S.), Institute for Laboratory Animal Research (U.S.), National Academies Press (U.S.) (2011). *Guide for the care and use of laboratory animals*. 8th ed. National Academies Press. https://nap.nationalacademies.org/catalog/12910/guide-for-the-care-and-use-of-laboratory-animals-eighth

Newman, M. C. (2020). *Fundamentals of ecotoxicology: The science of pollution*. 5th ed. CRC. https://doi.org/10.1201/9781351133999

Newsted, J. L., Beach, S. A., Gallagher, S. P., & Giesy, J. P. (2006). Pharmacokinetics and acute lethality of perfluorooctanesulfonate (PFOS) to juvenile mallard and northern bobwhite. *Archives of Environmental Contamination Toxicology*, *50*, 411–420. https://doi.org/10.1007/s00244-005-1137-x

Organisation for Economic Co-operation and Development (OECD). (2010). Test No. 317: Bioaccumulation in terrestrial oligochaetes. OECD (OECD Guidelines for the Testing of Chemicals, Section 3). https://www.oecd.org/en/publications/test-no-317-bioaccumulation-in-terrestrial-oligochaetes_9789264090934-en.html.

Organisation for Economic Co-operation and Development (OECD). (2012). Test No. 305: Bioaccumulation in fish: Aqueous and dietary exposure. OECD (OECD Guidelines for the Testing of Chemicals, Section 3). https://www.oecd-ilibrary.org/environment/test-no-305-bioaccumulation-in-fish-aqueous-and-dietary-exposure_9789264185296-en.

Organisation for Economic Co-operation and Development (OECD). (2016). Test No. 222: Earthworm reproduction test (*Eisenia fetida/Eisenia andrei*). OECD (OECD Guidelines for the Testing of Chemicals, Section 2). https://www.oecd.org/en/publications/test-no-222-earthworm-reproduction-test-eisenia-fetida-eisenia-andrei_9789264264496-en.html.

Pandelides, Z., Conder, J., Choi, Y., Allmon, E., Hoskins, T., Lee, L., Hoverman, J., & Sepúlveda, M. (2023). A critical review of amphibian per- and polyfluoroalkyl substance ecotoxicity research studies: Identification of screening levels in water and other useful resources for site-specific ecological risk assessments. *Environmental Toxicology and Chemistry*, *42*, 2078–2090. https://doi.org/10.1002/etc.5695

R Core Team. (2024). R: A language and environment for statistical computing. https://www.R-project.org.

Rich, C. D., Blaine, A. C., Hundal, L., & Higgins, C. P. (2015). Bioaccumulation of perfluoroalkyl acids by earthworms (*Eisenia fetida*) exposed to contaminated soils. *Environmental Science & Technology*, *49*, 881–888. https://doi.org/10.1021/es504152d

Scearce, A. E., Goossen, C. P., Schattman, R. E., Mallory, E. B., & MacRae, J. D. (2023). Linking drivers of plant per- and polyfluoroalkyl substance (PFAS) uptake to agricultural land management decisions. *Biointerphases*, *18*, 040801. https://doi.org/10.1116/6.0002772

Soetaert, K., Petzoldt, T., & Setzer, R. W. (2010). Solving differential equations in *R* : Package deSolve. *Journal of Statistical Software*, *33*, 1–25. https://doi.org/10.18637/jss.v033.i09

Soetaert, K., & Petzoldt, T. (2010). Inverse modelling, sensitivity and Monte Carlo analysis in *R* using package FME. *Journal of Statistical Software*, *33*https://doi.org/10.18637/jss.v033.i03http://www.jstatsoft.org/v33/i03/.

Sun, J. M., Kelly, B. C., Gobas, F. A. P. C., & Sunderland, E. M. (2022). A food web bioaccumulation model for the accumulation of per- and polyfluoroalkyl substances (PFAS) in fish: How important is renal elimination? *Environmental Science: Processes and Impacts*, *24*, 1152–1164. https://doi.org/10.1039/D2EM00047D

Tarazona, J. V., Rodríguez, C., Alonso, E., Sáez, M., González, F., San Andrés, M. D., Jiménez, B., & San Andrés, M. I. (2015). Toxicokinetics of perfluorooctane sulfonate in birds under environmentally realistic exposure conditions and development of a kinetic predictive model. *Toxicology Letters*, *232*, 363–368. https://doi.org/10.1016/j.toxlet.2014.11.022

Tarazona, J. V., Rodríguez, C., Alonso, E., Sáez, M., González, F., San Andrés, M. D., Jiménez, B., & San Andrés, M. I. (2016). Toxicokinetics of perfluorooctane sulfonate in rabbits under environmentally realistic exposure conditions and comparative assessment between mammals and birds. *Toxicology Letters*, *241*, 200–206. https://doi.org/10.1016/j.toxlet.2015.11.002

Tomy, G., Halldorson, T., & Tittlemier, S. (2005). Methanolic extraction of poly- and perfluorinated alkyl acids from biota. *Organohalogen Compounds*, *67*, 787–789. https://dioxin20xx.org/wp-content/uploads/pdfs/2005/05-494.pdf

U.S. Environmental Protection Agency. (2002). Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (Report No.: EPA-821-R-02-012). https://www.epa.gov/sites/default/files/2015-08/documents/acute-freshwater-and-marine-wet-manual_2002.pdf.

U.S. Environmental Protection Agency. (1993). Wildlife exposure factors handbook (Report No.: EPA/600/R-93/187). https://assessments.epa.gov/risk/document/&deid=2799

Weir, S. M., Youssif, M. R., Anderson, T., & Salice, C. J. (2023). Current progress in developing standardized methods for reptilian toxicity testing to inform ecological risk assessment. In G Liwszyc, ML Larramendy, Eds. *Bird and reptile species in environmental risk assessment strategies*. Royal Society of Chemistry. p. 130–150. https://books.rsc.org/books/book/2065/chapter/7522775/Current-Progress-in-Developing-Standardized

Willson, J. D., Hopkins, W. A., Bergeron, C. M., & Todd, B. D. (2012). Making leaps in amphibian ecotoxicology: translating individual-level effects of contaminants to population viability. *Ecological Applications*, *22*, 1791–1802. https://doi.org/10.1890/11-0915.1

Willson, J. D., & Hopkins, W. A. (2013). Evaluating the effects of anthropogenic stressors on source-sink dynamics in pond-breeding amphibians. *Conservation Biology*, *27*, 595–604. https://doi.org/10.1111/cobi.12044

Zhao, S., Zhu, L., Liu, L., Liu, Z., & Zhang, Y. (2013). Bioaccumulation of perfluoroalkyl carboxylates (PFCAs) and perfluoroalkane sulfonates (PFSAs) by earthworms (*Eisenia fetida*) in soil. *Environmental Pollution*, *179*, 45–52. https://doi.org/10.1016/j.envpol.2013.04.002

Zodrow, J. M., Frenchmeyer, M., Dally, K., Osborn, E., Anderson, P., & Divine, C. (2021). Development of per and polyfluoroalkyl substances ecological risk-based screening levels. *Environmental Toxicology and Chemistry*, *40*, 921–936. https://doi.org/10.1002/etc.4975

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