1	Title
2	A meta-analysis reveals PFAS concentrations double
3	with each trophic level across aquatic and terrestrial food
4	webs
5	Short title
6	PFAS biomagnification in food webs.
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45 Abstract

46	Per- and polyfluoroalkyl substances (PFAS) threaten ecosystems worldwide due to
47	their persistence, bioaccumulation, and toxicity. Through a global-scale meta-analysis
48	of 122 aquatic and terrestrial food webs from 64 studies, we analyse 1,009 trophic
49	magnification factors (TMFs) for 72 PFAS and identify key variability drivers. PFAS
50	concentrations systematically doubled with each trophic level increase (mean
51	TMF=2.00, 95% CI:1.64-2.45), confirming widespread biomagnification across
52	ecosystems. Methodological disparities across studies emerged as the dominant
53	source of TMF variability. Our models explained 84% of the variation in TMFs,
54	underscoring predictive capacity. Notably, the industrial alternative F-53B exhibited
55	the highest magnification (TMF=3.07, 95% CI:2.41-3.92), a critical finding given its
56	expanding use and minimal regulatory scrutiny. This synthesis establishes PFAS as
57	persistent trophic multipliers and provides a framework to prioritise high-risk
58	compounds and harmonise biomagnification assessments. Our results call for
59	consideration of stricter PFAS regulation to curb cascading ecological and health
60	impacts.

61 Teaser

A global analysis reveals "forever chemicals" levels double with each food chain step,
threatening ecosystems and human health.

64 MAIN TEXT

65 Introduction

Human activities increasingly destabilise ecological networks, eroding the integrity of 66 food webs and their capacity to withstand environmental shifts (1). This degradation 67 accelerates biodiversity decline and amplifies vulnerabilities across ecosystems, with 68 contamination by persistent toxic chemicals representing a pervasive and escalating 69 threat (2). Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals 70 specifically engineered for durability. PFAS are currently used across more than 200 71 categories of products (3), and their resistance to degradation has led to global 72 73 environmental infiltration, permeating ecosystems from industrial zones to remote habitats (4, 5). A portion of this contamination transfers from the geosphere to the 74 biosphere (6, 7) and moves from prey to predator (8). Once introduced into food 75 webs, PFAS can traverse trophic levels if organisms absorb these compounds faster 76 77 than they can metabolise or excrete them. Such dynamics drive trophic magnification, concentrating PFAS in apex predators, including humans, at levels exponentially 78 exceeding environmental background concentrations (9). Such type of 79 bioaccumulation, coupled with PFAS' known toxicity (10), risks destabilising 80 ecological hierarchies and exacerbating health crises across species, underscoring an 81 urgent need to quantify and mitigate their cascading impacts. 82 83 Efforts to quantify PFAS' impacts face the critical barrier of stark inconsistencies in reported trophic magnification (11, 12). Conflicting evidence ranges from reports of 84 85 negligible accumulation or even biodilution in select food webs (13, 14) to extreme biomagnification in others (9, 15, 16), with magnitudes varying over tenfold. This 86 87 unresolved variability hinders predictive models and regulatory decisions as 88 explanations remain contested. Competing hypotheses attribute discrepancies to inherent ecological complexity (e.g., food web structure, compound-specific traits) or 89 methodological artifacts that artificially inflate variability. Resolving this ambiguity is 90 91 essential to isolate true ecological risks from study design biases, a prerequisite for 92 evidence-based policy. To quantify PFAS biomagnification and resolve persistent ambiguities, we conducted 93 94 the first global meta-analysis integrating standardised trophic magnification factors

95 (TMFs) from 64 studies spanning 122 food webs. TMFs, calculated as the antilog of

96	log-concentration versus trophic-level regression slopes, provided a unified metric to
97	quantify cross-ecosystem trends. Our analysis systematically addressed four
98	objectives: [1] estimating PFAS and compound-specific TMFs, [2] dissecting within-
99	and between-study variability, [3] ranking drivers of variability (e.g., methodological,
100	ecological; Tab. 1), and [4] discussing critical data gaps. By synthesising fragmented
101	evidence into predictive models, this meta-analysis delivers definitive trophic
102	magnification values for PFAS as a class and individual compounds. Doing so
103	establishes a benchmark for harmonising future research and policy, bridging the
104	divide between ecological theory and actionable chemical regulation.

105

106 107 Tab. 1. Factors expected to have an impact on the trophic magnification estimate. The table provides the list of potential moderators (i.e., potential predictors of influence) alongside the prediction of the expected influence of each moderator, an explanation of the predictions, and references supporting the predictions. Moderators were chosen *a priori* and pre-registered in the research protocol (see (17)). The moderator "chemicals' regulation status" variable was added post-hoc; thus, it was 108 109 110

not pre-registered in the research protocol.

Moderator	Prediction	Explanation	References
Research methodological factors			
Whole- organism or organ/tissue- specific analysis	PFAS biomagnification estimates based exclusively on whole- organism samples may differ from those on a mix of whole-organism and organ-specific.	Whole-organism concentrations are usually measured at the base of food webs (plankton, invertebrates, and other small organisms). For animals of higher trophic levels (seals, bears, and other large organisms) practical and ethical reasons mean sampling is done on specific organs or fluids instead. The sampling strategy also pertains to whether the data was initially gathered due to a study's focus on ecological risk (whole animals) or human health risk (emphasizing edible parts such as fillets, eggs, muscle tissue, etc.).	(11, 12, 18)
PFAS concentrations normalised to lipid or protein levels	The biomagnification estimates of TMFs based on protein- or lipid- normalized PFAS concentrations may differ from those based on non- normalised concentrations.	Evaluation of biomagnification using TMFs based on protein- or lipid-normalized concentrations in food web organisms has been rarely suggested. However, it should be considered a source of variability in biomagnification estimates.	(11, 19)

Treatment of concentrations below analytical quantification or reporting limits	A substitution of undetected compound values by one-half of the limit value decreases the TMF compared with other approaches.	Using one-half of the limit value as a substitution for undetected compounds may inflate baseline compound concentrations.	(12, 18)
N-isotope trophic enrichment factor (TEF)	Different TEF choices can result in under- or overestimation of PFAS biomagnification.	Different studies use different N-isotope TEF to calculate the trophic level of species. The choice of TEF can affect the resulting TMF.	(12, 20, 21)
	Biological and	l environmental factors	
Sampling Latitude	PFAS biomagnification estimates for food webs closer to the equator may be lower than those at higher latitudes.	Tropical food webs are more intricate due to higher biodiversity, enabling more diverse consumer diets. Greater biomass and tissue turnover may dilute pollutants across networks. The latitude may be linked to the synergic effect of several factors, such as food web length and nature of top predator and food web baseline organism.	(12, 18, 22–24)
Type of breathing	PFAS biomagnification estimates tend to be higher in food webs that include solely air- breathing organisms or a combination of air breathing and water breathing organisms, compared to those consisting exclusively of water-breathing organisms.	Biomagnification of PFAS in food webs dominated by water breathing organisms is lower than in food webs dominated by air breathers because of the differences in metabolic rates and lipid content between these two types of organisms.	(12, 18, 22)
The lowest trophic level measured	Analysing lower trophic level organisms is likely to affect biomagnification estimates.	Studies where a primary producer was used as the base of the food web are likely to have different biomagnification estimates compared to those with a primary consumer. Extending the food web to include lower trophic levels increases the baseline variability in contaminant concentrations, which can dilute or amplify	(12, 22)

		biomagnification estimates depending on the specific bioaccumulation dynamics at the base of the food web.	
The highest trophic level measured	The trophic position of the top predator will likely affect the biomagnification estimates.	Higher trophic-level organisms typically have higher lipid contents and longer lifespans. These allow more bioaccumulation over time, leading to higher concentrations in the top predators.	(18, 22)
Food web length	A broader range in studied trophic levels will likely increase the biomagnification estimate.	Bioaccumulation occurs at each trophic transfer. With more trophic levels, contaminants accumulate to higher concentrations at the top predators.	(12, 18, 23)
	Compounds' pl	hysicochemical properties	
Carbon chain length	Biomagnification estimates tend to increase with the carbon chain length of PFAS.	Laboratory studies in which fish were exposed to contaminants solely through diet observed a direct positive relation between biomagnification factors and the number of carbon atoms.	(25–27)
Chemical functional group	Perfluoroalkyl sulfonates (PFSA) are more bioaccumulative in food webs than perfluoroalkyl carboxylic acids (PFCAs) of the same fluorinated carbon chain length.	There is no clear mechanistic explanation for the greater bioaccumulation potential of acids containing a sulfonyl functional group. Nevertheless, Jones et al. (2003) showed that sulfonic acids bind strongly to proteins and thus could show higher bioaccumulative potential.	(25–27)
		Others	
Chemicals' regulation status (added post-hoc)	PFAS listed in major international regulations have larger trophic magnification factors.	Chemicals, whose production and use are strongly regulated by global treaties such as the Stockholm Convention on Persistent Organic Pollutants, have higher trophic magnification potentials.	NA

111 **Results**





123

124Fig. 1. World map showing the geographical distribution of the 122 food webs included in the meta-analysis.125Each point represents a food web, with colours indicating the corresponding ecosystem type. A slight jitter was126applied to minimise overlap between points for clearer visualisation (for exact geographical locations, see Data127S2).

Ecosystem type:
 Aquatic
 Terrestrial

128 **PFAS trophic magnification**

129	Our multilevel meta-analytic model revealed an overall positive and statistically
130	significant TMF of 2 (TMF = 2.00 , 95% confidence interval (hereafter CI) = $(1.64$,
131	2.45); Fig. 2A). This indicates that, on average, the concentration of PFAS doubles

with each increase in trophic level when combining all studies, food webs, andchemicals included in the analysis.

- We observed a high level of relative heterogeneity (28) (i.e., the percentage of 134 variance between effect sizes that cannot be attributed to sampling error) across our 135 dataset ($I_{total}^2 = 97.55\%$), with the majority of the variation attributed to differences at 136 the effect size, study, and chemical levels ($I_{PFAS}^2 = 29.84\%$; $I_{study}^2 = 27.64\%$; $I_{es}^2 =$ 137 27.18%). A smaller proportion of the heterogeneity was associated with the food web 138 level ($I_{fw}^2 = 12.89\%$). To explain this heterogeneity and explore potential sources of 139 variability (Tab. 1), we conducted single- and multi-moderator meta-regression 140 141 analyses.
- Meta-regression analysis with PFAS identity as moderator identified PFAS type as a 142 143 statistically significant predictor of TMF ($F_{(df1 = 52, df2 = 935)} = 19.3$, p < 0.0001). Twelve PFAS exhibited results significantly greater than 1, with F-53B, PFOS, and PFDA 144 having the highest TMFs (F-53B: TMF = 3.07, CI = (2.41, 3.92); PFOS: TMF = 3.02, CI = 145 (2.64, 3.46); PFDA: TMF = 2.80, CI = (2.35, 3.33); PFUnDA: TMF = 2.41, CI = (2.04, 2.86); 146 PFNA: TMF = 2.21, CI = (1.85, 2.65); PFTrDA: TMF = 2.04, CI = (1.58, 2.64); PFDoDA: 147 TMF = 2.01, CI = (1.75, 2.32); FOSA: TMF = 1.89, CI = (1.38, 2.59); PFHxS: TMF = 1.76, 148 149 CI = (1.33, 2.32); PFTeDA: TMF = 1.42, CI = (1.15, 1.75); Fig. 2B; for a glossary of PFAS acronyms, see Data S3). Ten additional compounds also showed TMFs significantly 150 151 above 1 (Fig. S1), although these results were based on fewer than ten effect sizes. We found no statistical evidence of biodilution for any PFAS (i.e., TMF < 1; Fig. S1). 152 153 Notably, all TMFs for the substance F-53B were derived from food webs located in East Asia (Tab. S2), and we observed a significant effect of geographic regions (i.e., 154 North America, Europe, East Asia, and polar regions) on the TMF ($F_{(dfl = 5, df2 = 966)}$ = 155 9.3, p < 0.0001; Fig. S2). Stratifying by ecosystem type (terrestrial vs aquatic), we did 156 not find statistically significant differences in the results between these two ecosystem 157 types (F(df1 = 1, df2 = 1007) = 0, p = 0.8428; Fig. S3). 158





160	Fig. 2. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS) in food webs. (A)
161	Overall TMF based on a meta-analysis of 1,009 effect sizes from 117 aquatic and terrestrial food webs. The mean
162	meta-analytic estimate is represented by a black circle filled with red. The thicker bars indicate the 95%
163	confidence interval, while the thinner bars represent the 95% prediction interval. Light grey circles depict
164	individual effect sizes scaled by precision (inverse of the standard error, as shown in the legend). The number of
165	effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted
166	line highlights a TMF of 1 (biomagnification above 1 and biodilution below 1). The x-axis was capped at 10 for
167	improved visual readability and does not show 15 effect sizes (for the full version of the plot, see Fig. S4). (B)
168	Compound-specific TMFs for individual PFAS. A black bubble represents the mean TMF for individual chemicals,
169	and the bars indicate the 95% confidence interval. Bubble size represents the number of effect sizes contributing to
170	the estimate. Bubbles and k values in black represent estimates significantly different from 1 (i.e., $p < 0.05$). Dark
171	and light green shields identify compounds listed in the global treaty the Stockholm Convention on Persistent
172	Organic Pollutants and the European regulatory framework REACH regulation, respectively (for more information
173	on PFAS regulation classification, see the 'Statistical modelling overview' paragraph in the Methods section and
174	Tab. S3). Only the results for compounds with at least ten effect sizes are shown in panel B (for the full version of
175	the plot, see Fig. S1).

176 Sources of variability

177	Research methodological factors
178	A single-moderator meta-regression analysis revealed that the type of sample
179	analyzed (whole-organism, tissue-specific, or a combination of both) was a
180	statistically significant predictor of TMF ($F_{(df1 = 3, df2 = 1006)} = 20.9, p < 0.001$; Fig. 3A).
181	On average, TMFs calculated using tissue-specific samples (e.g., liver, muscle, blood
182	plasma) or whole-organism homogenates were 50% higher than those based solely on
183	whole-organism samples (TMF _{contrast} = 1.50 , CI = (1.21 , 1.84)). However, only 10%
184	of studies $(n = 6)$ applied a biomass conversion to adjust tissue-specific
185	concentrations to whole-organism equivalents.
186	We also found significant differences between TMFs derived from non-normalized
187	PFAS concentrations and those adjusted for protein or lipid content ($F_{(df1 = 1, df2 = 943)} =$
188	17.5, $p < 0.0001$; Fig. 3B). TMFs based on non-normalized concentrations were, on
189	average, 44% higher than those using normalized values (TMF _{contrast} = 1.44, CI =
190	(1.21, 1.70), $p < 0.0001$). Additionally, the nitrogen isotope trophic enrichment factor
191	(TEF) $(F_{(df1 = 4, df2 = 735)} = 12.1, p < 0.0001; Fig. 3C)$ and the method used to handle
192	undetected data ($F_{(df1 = 1, df2 = 5)} = 4$, p = 0.0014; Fig. 3D) were also significant
193	predictors of TMF. We observed high variability in how undetected data were treated
194	across studies (Tab. S4).



196 Fig. 3. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS), stratified by sample 197 type (A), concentration determination method (B), trophic enrichment factor (C), and treatment strategy of 198 undetected values (D). The meta-analytic mean estimate for each stratification is represented by a red-filled circle 199 with a black outline. Thicker bars denote the 95% confidence interval, while thinner bars represent the 95% 200 prediction interval. Light grey circles show individual effect sizes, scaled by precision (inverse of the standard error, as indicated in the legend). The number of effect sizes is represented by "k," while the number in parentheses 201 202 indicates the number of studies. The red dotted line marks a TMF of 1, with values above 1 indicating 203 biomagnification and those below 1 indicating biodilution. TMF values are capped at 14 for improved visual

clarity (refer to Fig. S5-S8 for full versions). In panel A, "mixed" refers to a combination of specific tissue and
 whole-organism samples. R² values represent the proportion of variance explained only by the fixed effects in the
 model (marginal R²).

207 Biological, environmental, and chemical factors

- 208 We observed statistically significant differences in TMFs between food webs
- 209 composed exclusively of water-breathing organisms and those that included both
- 210 water- and air-breathing organisms ($F_{(df1 = 1, df2 = 1007)} = 6.2, p = 0.0128$; Fig. 4A).
- 211 Specifically, TMFs in food webs consisting only of water-breathing organisms were
- 212 52% lower (TMF_{contrast} = 0.52, CI = (0.31, 0.87)), on average, than those in mixed
- 213 food webs. Similarly, food webs with a water-breathing top predator had TMFs that
- 214 were 60% lower than those with an air-breathing top predator ($F_{(df1 = 1, df2 = 856)} = 6.6$, p

215 =
$$0.0104$$
; TMF_{contrast} = 0.60 , CI = (0.41, 0.88); Fig. 4B).

- 216 Our meta-regression analysis also identified PFAS chemical class as a moderator of
- 217 TMF ($F_{(df1 = 4, df2 = 973)} = 1.4$, p < 0.0001; Fig. 4C). In contrast, latitude showed no effect
- 218 on TMFs ($F_{(dfl = 1, df2 = 1007)} = 0, p = 0.9722$; Fig. 5A).





- 236 When we tested the regulation status of chemicals (i.e., whether they are listed under
- 237 the Stockholm Convention on Persistent Organic Pollutants, the REACH regulation,
- or remain internationally unregulated) as a post-hoc moderator (i.e., not pre-
- 239 registered; see Tab. S3 for details) we found an effect on TMF $(F_{(df1 = 3, df2 = 841)} = 11, p$
- 240 < 0.0001; Fig. S12). However, TMFs did not differ between internationally
- 241 unregulated compounds and those listed under REACH (TMF_{contrast} = 0.67, CI =
- 242 (0.39, 1.13)) or the Stockholm Convention ($TMF_{contrast} = 0.88, CI = (0.52, 1.49)$).





244Fig. 5. The relationship between trophic magnification of per- and polyfluoroalkyl substances (PFAS) and food245webs' latitude (A), trophic position of the food web's baseline organism (B), trophic position of the food web's

246 top predator (C), the number of trophic levels in the food web (D), PFAS carbon chain length (E), and 247 publication year of the included studies in the meta-analysis (F). The uni-moderator fitted models are depicted as 248 thick black lines, with their 95% confidence intervals shown as red dashed lines and 95% prediction intervals 249 represented by dotted black lines. Light grey circles represent the individual effect sizes (k), and the size of each 250 circle reflects its precision (inverse of standard error). The number of effect sizes is represented by "k," while the 251 number in parentheses indicates the number of studies. The TMF is on the natural logarithm scale to enhance 252 visual readability of results. R² values represent the proportion of variance explained only by the fixed effects in 253 the model (marginal R^2).

254 The full model and multi-model inference

The results from the multi-moderator meta-regression model (hereafter the "full 255 model"), which accounts for potential confounding correlations among moderators, 256 corroborated the findings of the univariate models, identifying sample type (i.e., 257 whole-body, tissue-specific, or mixed) and concentration determination method 258 (normalisation) as predictors of variability in TMF (p < 0.0001 for both). However, 259 unlike the univariate models, the full model revealed a borderline non-significant 260 effect of breathing type at both the food web and top predator levels (p = 0.051 and p 261 = 0.057, respectively). PFAS chemical class, carbon chain length, and food webs' 262 latitude did not emerge as predictors of change from the full model. We excluded five 263 moderators with moderate to high levels of missing data from the full model, 264 including the strategy for handling undetected values (n = 281), trophic enrichment 265 factor (n = 83), trophic levels of the baseline organism (n = 119) and top predator (n = 119)266 119), and food web length (n = 119) to preserve statistical power. A correlation 267 analysis, aided by visual inspection of an alluvial plot of categorical variables, 268 confirmed that the included moderators were not highly correlated (i.e., no evidence 269 of excessive collinearity; Fig. S13). Notably, the full model accounted for 84% of the 270 variation in the dataset ($R^2 = 0.837$). 271

We used multi-model inference to generate models with all possible combinations of 272 moderators from the full model. Two "best models" were identified based on the 273 lowest Akaike Information Criterion (AIC). Sample type, concentration determination 274 method, breathing type of top predator and whole food web, and PFAS carbon chain 275 length and chemical class appear in both models. Food webs' latitude did not appear 276 in the second-ranked model but appeared in the first-ranked. Relative importance 277 analysis with Akaike weights identified (1) the type of sample, concentration 278 determination method, and carbon chain length as the most important predictors of 279

change, (2) the breathing type of top predator and whole food web and PFAS
chemical class as secondary predictors, and (3) food webs' latitude as the least
important predictor (Fig. S14).

283 **Publication bias and sensitivity analysis**

A visual assessment of study precision (inverse of standard error, Fig. S15) and a 284 meta-regression of time-lag ($F_{(df1 = 1, df2 = 1007)} = 0$, p = 0.9490; Fig. 5F) provided little 285 evidence of publication bias. However, the meta-regression with standard error as a 286 moderator indicated a potential publication bias ($F_{(df1 = 1, df2 = 1007)} = 22$, p < 0.001; Fig. 287 S16). Applying a two-step robust point and variance estimation (29) reduced the 288 289 effect magnitude by a factor of 3.46, but the direction and statistical significance remained unchanged (TMF = 1.65, CI = (1.28, 2.13)). The leave-one-out analysis 290 showed that no individual study had a substantial impact on the overall results (Fig. 291 S17). A validation test of the meta-regression model, using PFAS identity as a 292 293 moderator, found no evidence of overparameterisation, supporting the model's reliability (Method S1; Fig. S18). A study validity assessment was performed using a 294 modified version of SYRCLE's risk of bias tool (30) (Method S2; Data S5). 295 Excluding studies with at least one high-risk-of-bias item did not change the overall 296 direction of the meta-analytic result (Fig. S19). It only slightly affected its magnitude, 297 298 providing evidence of no significant impact of the removal of potentially biased studies on the robustness of the findings. 299

300 Discussion

We found strong evidence for the amplification of PFAS contamination as it moves up 301 the food chain. Notably, when aggregating results from different chemicals (n = 75)302 and ecosystems (n = 122), we found that, on average, PFAS concentrations doubled 303 304 with each increase in trophic level. Our data showed high heterogeneity, with nearly 30% of TMF variability attributed to compound-specific differences. The rest was 305 evenly divided between within- and between-study factors. Methodological 306 differences across studies emerged as the primary drivers of TMF variation. Although 307 some differences reflect genuine biological variability, much of the observed variation 308 arises from inconsistent methodological decisions. Two earlier literature reviews (11, 309 12) hypothesised that multiple factors influence PFAS TMFs. Our analysis ranks the 310 311 contribution of these factors for the first time, using a quantitative approach and a model that explained 84% of the total variability in the data. 312

313 Sample type was one of the most important predictors of change in TMF estimates. Specifically, TMFs measured in food webs where lower trophic level organisms were 314 315 analysed as whole organisms and upper trophic level ones using a tissue-specific sample had a 50% higher TMF than those with all organisms analysed as whole 316 organisms. This effect arises because some tissues and organs, such as the liver, 317 318 muscle, and lung, accumulate the highest PFAS concentrations (31, 32), resulting in an overestimation of the TMF. Conversely, using tissues not prioritised by PFAS 319 bioaccumulation (e.g., non-target organs) in top predators risks underestimating 320 TMFs, obscuring true magnification trends. Our finding quantitatively supports 321 concerns previously raised by a literature review (18) and emphasises the impact of 322 sampling strategies on TMF outcomes. Concentration determination methods also 323 324 contributed to variability, as normalising PFAS concentrations for lipid or protein content consistently resulted in lower TMFs. While a previous study (19) first 325 proposed accounting for these factors, our analysis reinforces the importance of 326 recognising lipid- and protein-normalization as critical sources of variation in TMF 327 328 calculations. Furthermore, the observed influence of nitrogen isotope enrichment factors (TEF) on TMF reflects the significance of accurately quantifying Δ^{15} N 329 dynamics. While the widely applied average $\Delta^{15}N$ of 3.4% per trophic level offers 330 practical utility, its oversimplification risks misrepresenting food web structure by 331 332 masking taxon-specific variability and the dynamic nature of isotopic discrimination

(20). However, our model revealed that the specific contribution of TEF to TMF
variability was relatively little when accounting for the influence of other moderators
included in this meta-analysis. We also observed an effect of the strategy used to deal
with undetected values (e.g., the instrument limit divided by two) on the TMF.
However, the high variability of strategies and missing information hindered our
ability to test its effect while controlling for other moderators.

Beyond methodological drivers, true biological variability associated with ecological 339 340 and environmental differences exerts limited influence. While TMFs were 341 significantly higher in food webs containing both water and air breathing organisms, 342 particularly those culminating in air-breathing apex predators, this pattern is likely attributable to confounding factors inherent to the samples. Notably, when accounting 343 344 for potential confounders, biological variability did not emerge as a robust predictor of observed differences. A plausible explanation for this confounding lies in the 345 346 structural composition of such food webs: systems integrating both water and air breathing organisms predominantly terminate in air breathing predators, where PFAS 347 concentrations are measured in specific tissues or organs. This measurement focus 348 may inadvertently conflate biological processes with methodological artifacts tied to 349 tissue-specific PFAS bioaccumulation dynamics. Furthermore, we observed no 350 significant differences in TMFs between terrestrial and aquatic food webs, 351 corroborating the hypothesis of the aforementioned confounding effect. 352

Building on these general patterns, our analysis revealed significant trophic 353 magnification for twelve individual compounds. Among these, F-53B (including 6:2 354 355 and 8:2 Cl-PFESA), PFOS (including linear and branched isomers), and PFDA exhibited the highest magnification factors (F-53B: TMF = 3.07, CI = (2.41, 3.92); PFOS: 356 357 TMF = 3.02, CI = (2.64, 3.46); PFDA: TMF = 2.80, CI = (2.35, 3.33)). Of these twelve compounds, six are currently regulated under a global treaty, the Stockholm 358 359 Convention on Persistent Organic Pollutants, eight are listed in the European REACH 360 regulation, and one (F-53B) remains unregulated at the international level (33). We observed significant variation in TMF across compounds, with some exhibiting 361 particularly high magnification patterns, warranting closer examination. Notably, our 362 363 findings reveal that F-53B, a trade name for a complex mixture of chlorinated polyfluoroalkyl ether sulfonic acids (primarily 6:2 and 8:2 Cl-PFESA), exhibits a 364 higher trophic magnification factor than PFOS, the compound it was designed to 365

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replace (34). This finding raises concerns about the environmental safety of F-53B 366 and suggests it may qualify for classification as a very bioaccumulative (vB) 367 substance under the REACH regulation. Originally synthesized in the 1970s, 368 chlorinated polyfluoroalkyl ether sulfonic acids have been used predominantly as mist 369 suppressants in China's electroplating industry (35). Mist suppressants reduce the 370 371 formation of airborne droplets or fumes during industrial processes. Production of F-53B increased after 2000, following the phase-out of PFOS by major manufacturers 372 from 2000 to 2002 (36). However, some industries ceased F-53B production in 2020 373 374 due to more stringent environmental regulations (37). Like PFOS, F-53B is resistant to degradation and poses a risk to aquatic ecosystems (35). It has been widely 375 detected in the blood of the Chinese population, with several health disorders linked 376 to its exposure (33, 38). Although F-53B use remains largely confined to China, 377 environmental monitoring has detected its presence in wildlife and ecosystems there 378 (14, 34, 39), and, to a lesser extent, in South Korea (40), Greenland (41), the United 379 States, and Europe (42). Our results reiterate prior concerns (43) about F-53B's 380 extreme bio-persistence, highlighting its potential risks as one of the most enduring 381 PFAS compounds studied to date. 382

We acknowledge that our meta-analysis rests on key assumptions inherent to the use 383 of the TMF. First, the TMF assumes steady-state conditions (11), where the intake and 384 elimination of PFAS are balanced. If the included studies in our meta-analysis violate 385 this assumption, our findings may capture short-term fluctuations rather than long-386 term trends in PFAS TMF, potentially affecting its accuracy. Second, the TMF 387 assumes that dietary intake is the primary pathway of contaminant exposure, and that 388 trophic level largely determines contaminant buildup in organisms and food webs 389 390 (18). However, the relationship between chemical concentration and trophic level may be distorted due to variability within and between species if different exposure 391 392 pathways (e.g., inhalation, dermal exposure, direct uptake from water or sediments) 393 significantly influence contaminant levels in upper-trophic level organisms.

In addition to these foundational assumptions, our meta-analysis has minor limitations worth mentioning. Our dataset is skewed towards aquatic food webs, with terrestrial food webs being underrepresented. As a result, our findings are likely more applicable to aquatic ecosystems. Although we did not observe significant differences between the two types of food webs, we consider our results for terrestrial food webs to be 399 preliminary. Furthermore, the geographic distribution of available studies, with a disproportionate concentration in North America, Europe, and China, limits the global 400 applicability of our findings, particularly for understudied regions like the southern 401 hemisphere. Finally, our results provided some evidence of publication bias, 402 suggesting that smaller studies in our dataset tend to report larger effect sizes. 403 404 However, after statistically accounting for this correlation, the direction and significance of the effect remained unchanged, demonstrating the robustness of our 405 findings. This pattern may therefore be driven by unexplained heterogeneity rather 406 407 than a small-study effect (44). Despite these limitations, our meta-analysis provides the most comprehensive quantitative synthesis of PFAS TMFs to date, though its 408 conclusions should be interpreted with these caveats in mind. 409

410 Considering our findings, we propose two recommendations for future research on TMF estimation for chemicals in general, including but not limited to PFAS. First, 411 412 researchers could convert biomass to tissue-specific concentrations into whole-body concentrations (see (45)) to improve the comparability of lower and higher trophic 413 levels. If a biomass conversion cannot be used, multiple tissues or organs should be 414 used for higher trophic level organisms. Small organisms like plankton and 415 invertebrates are typically analysed whole due to their size. In contrast, contaminants 416 in larger species (e.g., fish, birds, or mammals) are usually quantified via specific 417 tissues due to ethical and practical reasons, ignoring uneven accumulation in the body 418 (31, 46). Biomass-based conversion to whole-body concentrations or multi-tissue 419 analysis would improve TMF accuracy by accounting for relative heterogeneity. 420 Second, future studies should report TMFs using both protein-normalized and non-421 normalized concentrations. Unlike many persistent organic pollutants, PFAS 422 preferentially bind to proteins (47, 48), making dual reporting helpful for cross-423 chemical comparisons and standardised estimates across species with diverse tissue 424 425 compositions. Finally, studies should evaluate the sensitivity of their results to variations in the chosen TEF. In ecological food web models, TEF values are often 426 427 selected arbitrarily and may not accurately reflect the true isotopic enrichment per trophic level (20). Such discrepancies can influence the results, potentially leading to 428 429 overestimating or underestimating trophic position and biomagnification patterns. Our recommendations aim to enhance methodological consistency, reduce bias, and ensure 430 431 that observed TMF variability reflects true biological, chemical, or ecological

differences rather than methodological artifacts. For further guidance on measuring
the TMF, see (49).

In summary, our analysis provides compelling evidence of PFAS trophic 434 magnification in both aquatic and terrestrial food webs by an average factor of 2, 435 identifies F-53B and several other chemicals as highly biomagnifying, and highlights 436 methodological choices as key drivers of variability in TMF estimates. A TMF of 2 437 indicates that PFAS concentrations double at each trophic level, threatening apex 438 predators and humans and potentially destabilising biodiversity and food web 439 resilience. This quantifiable risk may demand urgent policy action: stricter regulation 440 of PFAS discharges, expanded monitoring of high-trophic species, and global treaties 441 to curb bio-accumulative chemical production. Furthermore, our results reveal 442 widespread methodological disparities that obscure true ecological drivers of PFAS 443 biomagnification, undermining risk assessments and delaying targeted regulations. 444 445 Addressing these inconsistencies must precede policy. Standardised protocols are essential to isolate real-world trends from study artifacts, ensuring regulatory 446 decisions reflect ecological reality. 447

449 Materials and Methods

Our methodology consisted of five key procedural steps. First, we registered the 450 project plan (17), which detailed the research questions, hypotheses, and methods. 451 Minor revisions were made to the original plan, and these changes were documented, 452 explained, and justified (Tab. S5). Second, we identified the research question 453 components (Method S3) and conducted a systematic literature search of primary 454 studies relevant to the research topic. Third, we extracted specific data items from the 455 literature and stored them in a relational database. Fourth, we tested our research 456 questions by extracting or estimating effect sizes and using statistical modelling 457 techniques. Fifth, we tested the robustness of our analysis through a publication bias 458 assessment and sensitivity analysis. The methods are presented in accordance with the 459 460 Method Reporting with Initials for Transparency (MeRIT) system (50), while data and analysis reporting adhere to the PRISMA-EcoEvo guidelines (51) (Tab. S6). We used 461 an adapted version of the SYRCLE's risk of bias tool for study validity assessment 462 (30) (see the Publication Bias and Sensitivity Analysis section for more details). The 463 464 raw data and code are publicly available in our GitHub repository

465 (<u>https://github.com/ThisIsLorenzo/PFAS_Trophic_Magnification</u>).

466 Systematic review and dataset structure

- LR conducted a systematic literature search across six academic databases (PubMed, 467 Web of Science Core Collection, Scopus, GreenFile via EBSCO, Bielefeld Academic 468 Search Engine, and ProQuest Theses & Dissertations) to identify studies on the 469 trophic magnification of PFAS. The Scopus search string was validated by cross-470 471 referencing 25 previously identified records from an earlier literature review (12), retrieving all entries, thereby confirming the string's comprehensiveness (Tab. S7). 472 473 The initial search yielded 3,744 bibliographic records. Comprehensive details regarding search dates, query syntaxes, and the number of hits per database are 474 provided in the Tab. S8. 475
- 476 Duplicated records were systematically identified and removed using a two-step
 477 process: first, through string-matching algorithms implemented in the R package
 478 synthesisr (52), which detected 1,385 duplicates; then, additional deduplication using
 479 Rayyan's proprietary function (https://www.rayyan.ai/), which identified 14 remaining
 480 duplicates. This resulted in a final corpus of 2,345 unique records.

Six independent reviewers (LR, ML, CW, PPottier, KM, PPollo) screened titles and 481 abstracts of 2,345 records against predefined eligibility criteria (Method S4). LR and 482 ML performed a pilot assessment on a 10% subset of records to ensure consistency in 483 full-text screening, after which LR completed the remaining full-text evaluations (Fig. 484 S20). Studies excluded during full-text screening were documented with rationale 485 (Tab. S9). LR extracted the data and organised it into five structured tables 486 summarising study characteristics, study validity assessment results, food web 487 parameters, PFAS analytes, and quantitative datasets used for effect size calculations 488 489 (Fig. S21).

490 The trophic magnification factor

In this meta-analysis, we used the Trophic Magnification Factor (TMF) as the effect 491 size, along with its standard error (SE), calculated as the square root of the sampling 492 variance. The TMF is commonly used to assess the trophic magnification potential of 493 494 pollutants and represents the increase in the concentration of a chemical compound per trophic level. The TMF is derived from the antilog of the slope (b in Equation 1) 495 of the relationship between log-transformed (to the base of 10 or Euler's number) 496 PFAS concentration and the trophic levels of organisms belonging to the same food 497 498 web (Equation 2).

499

500

 $TMF = 10^b$ or $TMF = e^b$

Equation 1

Equation 2

501

 $log_{10}[PFAS] = TL(b) + a$ or ln[PFAS] = TL(b) + a

502

503 where TL represents the trophic level (also known as trophic position) of organisms in 504 a food web and a is the intercept of the regression. The trophic level of an organism is 505 commonly calculated using nitrogen isotope analysis (Equation 3).

506
$$TL_c = \frac{(\delta^{15}N_c - \delta^{15}N_b)}{\Delta^{15}N + \lambda}$$

507

508 where TL_c refers to the trophic level of a consumer, $(\delta^{15}N_c - \delta^{15}N_b)$ is the 509 difference between the ratios of stable isotopes of nitrogen (i.e., ¹⁵N to ¹⁴N) in the

Equation 3

- 510 consumer and a baseline organism, $\Delta^{15}N$ represents the trophic discrimination factor 511 for $\delta^{15}N$ and λ is the trophic level of the baseline organism. The TMF is a reliable 512 and comparable method for evaluating PFAS transfer within food webs (12). It is 513 currently adopted under the REACH regulation as a metric of chemicals' 514 environmental persistence and long-term ecological impact (53).
- 515 In this meta-analysis, we directly extracted the TMF and its associated standard error 516 from the included studies. When the TMF or its standard error were not reported 517 directly, but necessary data were available, we calculated the TMF using the 518 calculation scenarios described in Method S5. When trophic levels were not reported, 519 but nitrogen isotope analysis data were available (i.e., $\delta^{15}N$) (Equation 4), we 520 employed these isotope results as a proxy for the trophic positions of organisms (54– 57).

$$log_{10}[PFAS] = \delta^{15}N(b) + a \text{ or } ln[PFAS] = \delta^{15}N(b) + a$$

Equation 4

524 where terms are as mentioned before.

525 Statistical modelling overview

522

523

To estimate the overall TMF for PFAS, LR employed a multilevel meta-analytic 526 527 model using the *rma.mv* function from the *metafor* R package (version 4.4.0) (58). This approach allowed for the incorporation of multiple sources of variability, as our 528 model accounted for random effects at four levels: between studies, between food 529 webs, between types of PFAS, and within studies. LR used the natural logarithm of 530 the TMF as the response variable and specified a variance-covariance matrix clustered 531 over food webs to account for dependence among effect sizes (59). The variance-532 covariance matrix was constructed using the squared standard error of the natural 533 logarithm of the TMF as the variance of the effect sizes. Additionally, a constant 534 within-study correlation coefficient of 0.5 was assumed. 535

536To assess and compare trophic magnification factors (TMFs) across individual PFAS537while controlling for covariates, we applied a subgroup-correlated effects meta-538regression model (60). This approach avoids assuming uniform biomagnification and539heterogeneity rates across all PFAS, enabling direct statistical comparison of TMFs540between compounds. By isolating compound-specific differences, we quantified their

relative biomagnification risks. LR built the model on a variance-covariance matrix
for each PFAS identity, incorporating the clustering effects from food webs and the
individual TMF identifiers. LR specified a random effects structure that allowed for
variability among food webs while treating the correlations among observations as
diagonal. We validated our models' overall quality and robustness to overparametrisation using their AICc value and profile likelihood of individual variance
components (Method S1; Fig. S18).

LR used uni-moderator meta-regression models to explore the moderating effect of 548 549 individual predictors on PFAS TMF. The models had each predictor as a fixed effect 550 (moderator) and the same random effect structure and variance-covariance matrix as the primary meta-analytic model. The multi-moderator meta-regression model (i.e., 551 552 full model) tested the combined effect of all moderators together. We assessed the moderators for missingness and correlation before fitting them into the full model 553 554 (Fig. S13). We also categorised chemicals according to their regulatory status. This classification was determined by evaluating whether the substances were included in 555 Annexes A, B, and C of the Stockholm Convention on Persistent Organic Pollutants, 556 listed in the European regulatory framework REACH regulation, or not listed in any 557 of these two international regulations (details provided in Tab. S3). The categorization 558 reflects the most accurate and comprehensive information available during the 559 analysis. We fitted the groups as moderators using the unregulated group as a 560 reference to see if unregulated compounds had a statistically different mean than 561 regulated ones. 562

563 Finally, to identify the most informative predictors of trophic magnification in our meta-analysis, LR employed model selection and multi-model inference using the 564 565 dredge function (MuMIn package, version 1.48.4) (61) on the full model. The dredge function systematically generated a set of candidate models by exploring all possible 566 567 combinations of predictor variables. These models were ranked based on Akaike Information Criterion corrected for small sample sizes (AICc), allowing us to assess 568 569 the relative support for each candidate model. We selected the top models with a delta AICc value of ≤ 4 for further analysis and interpretation and calculated the sum of 570 571 model weights for each predictor variable to estimate their relative importance.

All analyses were performed in the R computational environment (version 4.4.0) (62).
Confidence intervals (CIs) were estimated at the 95% level, and statistical
significance was determined at a p-value threshold of 0.05.

575 **Publication bias and sensitivity analysis**

- 576 LR assessed the risk of publication bias (63) by conducting the following two
- 577 analyses: 1) Visual inspection of the full model's residuals against their standard error
- 578 (44) and regression analysis of the effect size against its variance; 2) Regression
- analysis with publication year as moderator to test for time-lag bias (64). The
- robustness of the meta-analytic results was assessed through the following three
- 581 sensitivity analyses: 1) A 'Leave-one-out' analysis; 2) Exclusion of high-risk studies
- 582 according to a study validity assessment (*30*) (adapted SYRCLE's risk of bias tool;
- 583 Method S2); 3) Validation of the subgroup correlated effects model (Method S1).

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- 608Data and materials availability: Raw data and analysis code are available at the609provided GitHub link.

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Supplementary Materials for

A meta-analysis reveals PFAS concentrations double with each trophic level across aquatic and terrestrial food webs

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The PDF file includes:

- Supplementary Figures from Fig. S1 to S21
- Supplementary Text from Method S1 to S5
- Supplementary Tables from Tab. S1 to S9
- Legends for Data S1 to S5

Other Supplementary Material for this manuscript includes the following:

- Data S1 to S5.



Fig. S1. Trophic magnification of per- and polyfluoroalkyl substances (PFAS) in aquatic and terrestrial food webs. The plot is the full version of Figure 2 in the main text, showing the subgroup correlated effects model results for each PFAS regardless of their number of effect sizes.



Fig. S2. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS), stratified by geographic location of food webs. The meta-analytic mean estimate for each stratification is represented by a red-filled circle with a black outline. Thicker bars denote the 95% confidence interval, while thinner bars represent the 95% prediction interval. Light grey circles show individual effect sizes, scaled by precision (inverse of the standard error, as indicated in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line marks a trophic magnification factor (TMF) of 1, with values above 1 indicating biomagnification and those below 1 indicating biodilution. TMF values are capped at 12.5 for improved visual readability. The R^2 value represents the proportion of variance explained only by the fixed effect in the model (marginal R^2).



Fig. S3. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS), stratified by ecosystem type. The metaanalytic mean estimate for each stratification is represented by a red-filled circle with a black outline. Thicker bars denote the 95% confidence interval, while thinner bars represent the 95% prediction interval. Light grey circles show individual effect sizes, scaled by precision (inverse of the standard error, as indicated in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line marks a trophic magnification factor (TMF) of 1, with values above 1 indicating biomagnification and those below 1 indicating biodilution. TMF values are capped at 11 for improved visual readability. The R2 value represents the proportion of variance explained only by the fixed effect in the model (marginal R2).



Fig. S4. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS) in food webs. Overall TMF based on a meta-analysis of 1,009 effect sizes from 117 aquatic and terrestrial food webs. The mean meta-analytic estimate is represented by a black circle filled with red. The thicker bars indicate the 95% confidence interval, while the thinner bars represent the 95% prediction interval. Light grey circles depict individual effect sizes scaled by precision (inverse of the standard error, as shown in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line highlights a TMF of 1 (biomagnification above 1 and biodilution below 1).



Fig. S5. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS), stratified by sample type. The metaanalytic mean estimate for each stratification is represented by a red-filled circle with a black outline. Thicker bars denote the 95% confidence interval, while thinner bars represent the 95% prediction interval. Light grey circles show individual effect sizes, scaled by precision (inverse of the standard error, as indicated in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line marks a TMF of 1, with values above 1 indicating biomagnification and those below 1 indicating biodilution. "Mixed" refers to a combination of specific tissue and whole-organism samples. The R² value represents the proportion of variance explained only by the fixed effect in the model (marginal R²).



Fig. S6. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS), stratified by concentration determination method. The meta-analytic mean estimate for each stratification is represented by a red-filled circle with a black outline. Thicker bars denote the 95% confidence interval, while thinner bars represent the 95% prediction interval. Light grey circles show individual effect sizes, scaled by precision (inverse of the standard error, as indicated in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line marks a TMF of 1, with values above 1 indicating biomagnification and those below 1 indicating biodilution. The R² value represents the proportion of variance explained only by the fixed effect in the model (marginal R²).



Fig. S7. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS), stratified by trophic enrichment factor. The meta-analytic mean estimate for each stratification is represented by a red-filled circle with a black outline. Thicker bars denote the 95% confidence interval, while thinner bars represent the 95% prediction interval. Light grey circles show individual effect sizes, scaled by precision (inverse of the standard error, as indicated in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line marks a TMF of 1, with values above 1 indicating biomagnification and those below 1 indicating biodilution. The R^2 value represents the proportion of variance explained only by the fixed effect in the model (marginal R^2).



Fig. S8. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS), stratified by treatment stretgy of undetected values. The meta-analytic mean estimate for each stratification is represented by a red-filled circle with a black outline. Thicker bars denote the 95% confidence interval, while thinner bars represent the 95% prediction interval. Light grey circles show individual effect sizes, scaled by precision (inverse of the standard error, as indicated in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line marks a TMF of 1, with values above 1 indicating biomagnification and those below 1 indicating biodilution. The R² value represents the proportion of variance explained only by the fixed effect in the model (marginal R²).



Fig. S9. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS), stratified by food webs of exclusively water breathing organisms versus mixed breathing types. The meta-analytic mean estimate for each stratification is represented by a red-filled circle with a black outline. Thicker bars denote the 95% confidence interval, while thinner bars represent the 95% prediction interval. Light grey circles show individual effect sizes, scaled by precision (inverse of the standard error, as indicated in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line marks a TMF of 1, with values above 1 indicating biomagnification and those below 1 indicating biodilution. The R^2 value represents the proportion of variance explained only by the fixed effect in the model (marginal R^2).



Fig. S10. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS), stratified by food webs with either air breathing or water breathing top predators. The meta-analytic mean estimate for each stratification is represented by a red-filled circle with a black outline. Thicker bars denote the 95% confidence interval, while thinner bars represent the 95% prediction interval. Light grey circles show individual effect sizes, scaled by precision (inverse of the standard error, as indicated in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line marks a TMF of 1, with values above 1 indicating biomagnification and those below 1 indicating biodilution. The R² value represents the proportion of variance explained only by the fixed effect in the model (marginal R²).



Fig. S11. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS), stratified by PFAS chemical class. The meta-analytic mean estimate for each stratification is represented by a red-filled circle with a black outline. Thicker bars denote the 95% confidence interval, while thinner bars represent the 95% prediction interval. Light grey circles show individual effect sizes, scaled by precision (inverse of the standard error, as indicated in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line marks a TMF of 1, with values above 1 indicating biodilution. The R^2 value represents the proportion of variance explained only by the fixed effect in the model (marginal R^2).



Fig. S12. Trophic magnification factor (TMF) of per- and polyfluoroalkyl substances (PFAS), stratified by chemicals' international regulation status. 'SCPOPs' refers to PFAS listed under one of the annexes of the Stockholm Convention on Persistent Organic Pollutants. 'REACH' represents PFAS regulated within the European Union under the REACH framework. 'None' indicates PFAS that are not subject to any international regulation. The meta-analytic mean estimate for each stratification is represented by a red-filled circle with a black outline. Thicker bars denote the 95% confidence interval, while thinner bars represent the 95% prediction interval. Light grey circles show individual effect sizes, scaled by precision (inverse of the standard error, as indicated in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line marks a trophic magnification factor (TMF) of 1, with values above 1 indicating biomagnification and those below 1 indicating biodilution. TMF values are capped at 11 for improved visual readability. The R² value represents the proportion of variance explained only by the fixed effect in the model (marginal R²).



Fig. S13. Alluvial plot of the overlap among the three categorical variables included in the full model (see Methods). The plot visualises the interrelationships between the categorical variables, providing a clear overview of data distributions and overlaps. TMF frequency defines the number of TMFs. It serves as a tool to identify potential multicollinearity issues that may affect the full model.



Fig. S14. Relative importance of tested moderator variables based on Akaike weights, calculated from the Akaike Information Criterion (AIC). Importance was assessed across 256 candidate models by summing the Akaike weights of each moderator variable appearing in all models. These weights indicate the probability of a given candidate model being the best, derived from a Bayesian framework with a prior distribution. Additionally, the marginal R^2 (indicated in red) was estimated using a uni-moderator model, where each moderator variable served as the fixed effect, representing the proportion of variance explained. R^2 values represent the proportion of variance explained only by the fixed effects in the model (marginal R^2).



Fig. S15. Funnel plot for visual inspection of studies' precision. The plot shows the distribution of residual values of effect sizes in relation to their precision (inverse of standard error) from the full model (see the 'Statistical modelling overview' paragraph).



Fig. 16. Publication bias test. The plot presents the results of a meta-regression using the logarithmic standard error of lnTMF as a moderator. The regression suggests a moderate publication bias (F(df1 = 1, df2 = 1007) = 22, p < 0.001). To account for this, we applied a two-step robust point and variance estimation to evaluate whether the direction and magnitude of the effect changed significantly (see 'Publication bias and sensitivity analysis' section in the main text).



Fig. S17. Forest plot illustrating the results of leave-one-out sensitivity analyses. The vertical solid line represents the overall metaanalytic estimate, with the dotted lines marking its 95% confidence intervals. Each black point and solid line show the meta-analytic estimate and its confidence intervals, respectively, after excluding individual studies. The plot indicates that no single study significantly influenced the overall meta-analytic result.



Fig. S18. Model validation and profile likelihood for the 17 PFAS included in the subgroup-correlated effects model. Profile likelihood plots show how the log-likelihood changes with different values of the between-study variance for various PFAS in the model. The subgroup-correlated effects meta-regression model included PFAS identity as a fixed effect. The red dashed vertical lines represent the variance component relative to the individual PFAS, which resulted from a subgroup analysis, in which the dataset was restricted to observations related to each specific PFAS (see Method S1 for details). Clear peaks indicate a well-defined estimate for the between-study variance, and the maximum log-likelihood (highest point) suggests the best estimate of the between-study variance. If a profile is flat or constantly decreasing, the estimate of the between-study variance might be unreliable or close to zero, meaning there's little between-study variation.



Fig. S19. Overall TMF after filtering out studies flagged by the study validity assessment. The model excludes any study with at least one high-risk-of-bias item. The mean meta-analytic estimate is represented by a black circle filled with red. The thicker bars indicate the 95% confidence interval, while the thinner bars represent the 95% prediction interval. Light grey circles depict individual effect sizes scaled by precision (inverse of the standard error, as shown in the legend). The number of effect sizes is represented by "k," while the number in parentheses indicates the number of studies. The red dotted line highlights a TMF of 1 (biomagnification above 1 and biodilution below 1). The x-axis was capped at 10 for improved visual readability.



Fig. S20. Search and screening flow PRISMA diagram. The diagram illustrates the number of records at different stages of the selection process for studies incorporated in the meta-analysis.



Fig. 21. Diagram illustrating the structure of the relational database of the extracted data. The database consists of five interconnected tables linked through primary and foreign keys. TMF_data contains quantitative datasets for effect size calculations, PFAS_data includes details on PFAS analytes, fw_data represents food web parameters, study_data captures study characteristics, and RoB_data presents quality assessment results for study validity.

Supplementary Text

Method S1

We validated our subgroup-correlated effects meta-regression model (60) using a three-step approach to ensure the robustness and reliability of our variance estimates:

- Model fit comparison: We evaluated our model's relative fit by comparing its Akaike Information Criterion corrected for small sample sizes (AICc) with the AICc values of two alternative candidate models. We excluded models with higher AICc. A lower AICc value indicates a better balance between model fit and complexity, providing evidence that our model effectively captures key patterns in the data.
- 2) **Profile likelihood examination**: To assess whether variance components were wellestimated and consistent with model assumptions, we examined the profile likelihood for each individual PFAS. The profile likelihood plots, presented in Fig. S18, depict how the loglikelihood changes across different values of the variance parameter (τ^2). A well-defined peak suggests stable and identifiable variance estimates, whereas a flat or irregular likelihood profile could indicate estimation challenges or weak identifiability of variance parameters. The clear peaks observed in our plots suggest that our variance estimates are well-supported by the data.
- 3) **Subgroup analysis for variance components**: We further validated our variance estimates by comparing the profile likelihood for each individual PFAS to variance components derived from a subgroup analysis (red dashed lines in plots of Fig. S18), where we restricted the dataset to observations related to each specific PFAS. This step ensured that our model-derived variance estimates were consistent with those obtained from a more traditional subgroup analysis. By demonstrating alignment between the two approaches, we confirmed that the subgroup-correlated effects model appropriately accounts for within-PFAS variation while leveraging information across the full dataset.

We assessed the internal validity of the included studies using a modified version of SYRCLE's risk of bias tool for animal studies (Hooijmans et al., 2014). This tool was applied to each study to evaluate five types of bias: selection bias, performance bias, measurement bias, reporting bias, and funding bias. The table below defines each type of bias and outlines the key questions we considered to evaluate the potential risk of bias.

Type of bias	Definition	Evaluated criteria
Selection bias	Occurs when the sample selected for a study is	- All organisms were collected
	not representative of the population it is drawn	within an appropriate or
	from, leading to results that are not	consistent sampling period (e.g.,
	generalisable.	the same season).
		- Adequate number of trophic
		levels.
Measurement bias	Occurs when systematic differences exist in	- The study reports the TMF and
	how outcomes are measured or assessed	its error. If not, it provides data
	between studies.	for their calculation. High risk
		arises when we extract raw data
		from plots and calculate the
		TMF and its error by ourselves.
Reporting bias	Happens when certain results are selectively	- Is there evidence of incomplete
	reported based on their nature or direction, often	or selective reporting of results
	favoring statistically significant findings that are	(e.g., only statistically
	more likely to be published.	significant slopes)?
		- Are measured contaminant
		concentrations in biota samples
		above the detection limit? If
		not, does the study provide an
		appropriate strategy for
		handling undetected values?
Funding bias	Occurs when the source of funding influences	- Are there any apparent conflicts
	the study's design, conduct, analysis, or	of interest or undue influence
	reporting, often in ways that favor the sponsor's	from funding sources?
	interests.	

We defined the research question components according to the PICO/PECO framework (Richardson et al., 1995) as follows:

- Population: The population in this study refers to the global aquatic and terrestrial food webs (including plants and animals) affected by PFAS contamination.
- Intervention/Exposure: The exposure of interest is the exposure to any PFAS. Thus, PFAS contamination must be present and quantitatively measured in (whole or any part of) organisms within the studied ecosystem.
- Comparator: Not applicable. Instead, we will quantitatively assess the effects of factors that might influence PFAS trophic magnification. These factors include research methodologies, geographical variables, ecosystem characteristics, and food web composition (see Table 1 in the article).
- Outcome: The outcome of interest is the trophic magnification factor (TMF) within a studied food web. The TMF is the anti-log of the slope of the relationship between logarithm-transformed PFAS concentrations and trophic levels of biota within a food web.
- Study design: Any study design, such as field-based observational and mesocosms-based studies, is eligible. Studies must estimate and provide the trophic magnification factor or the trophic magnification slope.
- Other restrictions:
 - Time range: No restrictions.
 - Languages: Only studies published in English or a language spoken by any of the authors (i.e., Italian, Japanese, Polish, Russian, Traditional and Simplified Chinese, French, Portuguese, Spanish) will be eligible due to language constraints.

Eligibility criteria at the title plus abstract level:

- The study's title and, optionally, its abstract are available.
- The study is a peer-reviewed journal article, a pre-print, or a thesis (i.e., bachelor's thesis, master's thesis, doctoral and postdoctoral thesis).
- The study likely is an empirical study, not a review.
- The study likely quantified at least one PFAS concentration in more than two organisms in the same food web.

Eligibility criteria at the full-text level:

- The full text is available for examination and data extraction.
- The full text is written in English or any eligible language (i.e., Italian, Japanese, Polish, Russian, Traditional and Simplified Chinese, French, Portuguese, Spanish).
- The study is an empirical study.
- The study quantified at least one PFAS concentration in more than two organisms from the same food web.
- The study provided the trophic magnification factor (TMF) of at least one PFAS and its standard error or 95% confidence intervals. Alternatively, it must provide the trophic magnification slope (TMS) and its standard error or 95% confidence intervals. If neither TMF nor TMS values were provided, the study must report linear regression plots of PFAS concentrations versus trophic levels of organisms or nitrogen isotope analysis (proxy of trophic levels).

If the included studies did not provide the trophic magnification factor (TMF) and/or its standard error, we adopted the following calculation scenarios:

- The study provides the trophic magnification slope (TMS) and its standard error. We
 calculated the TMF and its standard error back-transforming the TMS by doing an anti-log of
 the slope and error.
- 2. The study provides the TMF and its 95% confidence intervals. We calculated the standard error by dividing the difference between the upper and lower confidence intervals by 1.96.
- 3. The study provides the TMF or TMS and the p-value. We calculated the standard error by dividing the TMS by the z-value. We calculated the z-value by taking the p-value, dividing it by 2, subtracting this result from 1, and then finding the corresponding z-value from the standard normal distribution.
- 4. The study provides a plot illustrating the regression between PFAS concentrations and trophic levels of organisms or stable nitrogen isotope analysis. We employed the *metaDigitise* R package (version 1.0.1) or its graphical user interface *shinyDigitise* (Ivimey-Cook et al., 2023) to extract the x- and y-axis coordinates (i.e., trophic levels of organisms or stable nitrogen isotope analysis and PFAS concentrations). Then, we run linear regression models to calculate the TMS and its error.

Supplementary Tables

Supplementary Table 1

Tab. S1. List of studies included in the meta-analysis.

Reference	Title	DOI
(19)	Perfluoroalkyl contaminants in an Arctic marine food web: trophic	10.1021/es9003894
	magnification and wildlife exposure	
(15)	Investigation of the spatial variability of poly-and perfluoroalkyl	10.1016/j.scitotenv.2019.05.461
	substance trophic magnification in selected riverine ecosystems	-
(65)	Trophic magnification and isomer fractionation of perfluoroalkyl	10.1021/es405018b
	substances in the food web of Taihu Lake, China	
(66)	Trophic Magnification of Legacy (PCB, DDT and Hg) and Emerging	10.3390/w12061591
	Pollutants (PFAS) in the Fish Community of a Small Protected	
	Southern Alpine Lake (Lake Mergozzo, Northern Italy)	
(67)	Biomagnification of perfluoroalkyl compounds in the bottlenose	10.1021/es060233b
	dolphin (Tursiops truncatus) food web	
(68)	Fractionation and bioaccumulation of perfluorooctane sulfonate	10.1021/es800906r
	(PFOS) isomers in a Lake Ontario food web	
(16)	Trophodynamics of some PFCs and BFRs in a western Canadian	10.1021/es900162n
	Arctic marine food web	
(69)	Fluorinated organic compounds in an eastern Arctic marine food web	10.1021/es049620g
(70)	Trophic magnification of poly-and perfluorinated compounds in a subtropical food web	10.1021/es200432n
(71)	Evidence for the trophic transfer of perfluoroalkylated substances in	10.1021/acs.est.7b02399
· ·	a temperate macrotidal estuary	
(72)	Biomagnification of perfluoroalkyl acids (PFAAs) in the food web of	10.1039/C9EM00322C
	an urban river: Assessment of the trophic transfer of targeted and	
	unknown precursors and implications.	
(73)	Bioaccumulation of per- and polyfluoroalkyl substances (PFASs) in	10.1016/j.scitotenv.2020.142146
	a tropical estuarine food web.	
(74)	Perfluoroalkyl contaminants in a food web from Lake Ontario.	10.1021/es049331s
(75)	Bioaccumulation and trophic transfer of perfluorinated compounds in a eutrophic freshwater food web.	10.1016/j.envpol.2013.09.011
(76)	Isomer-specific trophic transfer of perfluorocarboxylic acids in the	10.1021/es504445x
	marine food web of Liaodong Bay, North China.	
(77)	Distribution, bioaccumulation and trophic transfer of chlorinated	10.1016/j.envpol.2018.05.087
	polyfluoroalkyl ether sulfonic acids in the marine food web of Bohai,	
	China.	
(78)	Residues, bioaccumulations and biomagnification of perfluoroalkyl	10.1016/j.envpol.2018.05.001
	acids (PFAAs) in aquatic animals from Lake Chaohu, China.	
(79)	Occurrence and trophic transfer of per- and polyfluoroalkyl	10.1016/j.envpol.2019.113383
	substances in an Antarctic ecosystem.	10.1001/ 0.04505
(80)	Fluorinated precursor compounds in sediments as a source of	10.1021/acs.est.0c0458/
(01)	Occurrence activity acids (PFAA) to blota.	10 1016/:: 4-4 2018 02 201
(81)	legger per and polyflyoroolkyl substances in Taiby Lake. China	10.1016/j.scholenv.2018.03.301
(87)	Managing health risks of perfluoroalkyl acids in agustic food from a	10 1016/i envint 2020 105621
(02)	riverestuary, sea environment affected by fluorochemical industry	10.1010/j.envint.2020.105021
(83)	Bioaccumulation trophic transfer and biomagnification of	10 1016/i ibazmat 2020 124681
(03)	nerfluoroalkyl acids (PFAAs) in the marine food web of the South	10.1010/J.Jnazinat.2020.124081
	China Sea	
(84)	First report on the bioaccumulation and trophic transfer of	10.1021/acs.est.1c00965
	perfluoroalkyl ether carboxylic acids in estuarine food web.	

(14)	Legacy and alternative per- and polyfluoroalkyl substances in a subtropical marine food web from the Beibu Gulf, South China: Fate, trophic transfer and health risk assessment.	10.1016/j.jhazmat.2020.123618
(85)	Biomagnification and health risks of perflfluoroalkyl acids (PFAAs) in seafood from the Yangtze river estuary of China	10.1016/j.envpol.2023.122930
(86)	Chlorinated polyfluoroalkyl ether sulfonic acids in marine organisms from Bohai Sea, China: occurrence, temporal variations, and trophic transfer behavior	10.1021/acs.est.6b06593
(87)	Trophic magnification of short-chain per-and polyfluoroalkyl substances in a terrestrial food chain from the Tibetan Plateau	10.1021/acs.estlett.1c01009
(88)	Biomagnification of perfluorinated compounds in a remote terrestrial food chain: lichen-caribou-wolf	10.1021/es201353v
(89)	Comprehensive screening of polar emerging organic contaminants including PFASs and evaluation of the trophic transfer behavior in a freshwater food web	10.1016/j.watres.2022.118514
(90)	Bioaccumulation and Biomagnification of Perfluoroalkyl Substances (PFAS) in a Subarctic Ringed Seal Food Web in Lake Melville, Northern Labrador, Canada	Thesis
(91)	Ecological characteristics impact PFAS concentrations in a US North Atlantic food web	10.1016/j.scitotenv.2023.163302
(92)	Biomanipulation impacts on per-and polyfluoroalkyl substances accumulation and trophic transfer in an eutrophic lake	10.1016/j.envint.2021.107057
(93)	Food web on ice: A pragmatic approach to investigate the trophic magnification of chemicals of concern	10.1186/s12302-021-00530-x
(94)	Bioaccumulation of polyfluoroalkyl substances in the Lake Huron aquatic food web	10.1016/j.scitotenv.2022.152974
(95)	Bioaccumulation and trophic magnification of emerging and legacy per- and polyfluoroalkyl substances (PFAS) in a St. Lawrence River food web	10.1016/j.envpol.2022.119739
(96)	Sediment-seawater partitioning, bioaccumulation, and biomagnification of perfluorobutane sulfonamide in marine environment	10.1016/j.watres.2024.121466
(97)	Bioaccumulation and biomagnification of emerging poly-and perfluoroalkyl substances in marine organisms	10.1016/j.scitotenv.2022.158117
(98)	Legacy and Emerging Per- and Polyfluoroalkyl Substances in a Subtropical Marine Food Web: Suspect Screening, Isomer Profile, and Identification of Analytical Interference	10.1021/acs.est.3c00374
(99)	PFAS bioaccumulation in Antarctic breeding south polar skua (Catharacta maccormicki) and its prey items	Thesis
(100)	Perfluoroalkyl acids (PFAAs) in the aquatic food web of a temperate urban lake in East China: Bioaccumulation, biomagnification, and probabilistic human health risk	10.1016/j.envpol.2021.118748
(8)	Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web	10.1016/j.envpol.2006.09.021
(101)	Perfluorinated and Polyfluorinated Compounds in Lake Food Websfrom the Canadian High Arctic	10.1021/es5048649
(102)	PFAS accumulation in indigenous and translocated aquatic organisms from Belgium, with translation to human and ecological health risk	10.1186/s12302-021-00477-z
(103)	Bioaccumulation and trophic transfer of per- and polyfluoroalkyl substances in a subtropical mangrove estuary food web	10.1016/j.scitotenv.2024.172094
(104)	Bioaccumulation of perfluoroalkyl substances in the Lake Erie food web	10.1016/j.envpol.2022.120677
(105)	New insights from an eight-year study on per- and polyfluoroalkyl substances in an urban terrestrial ecosystem	10.1016/j.envpol.2024.123735
(106)	Uptake of Hydrophobic Organic Compounds, Including Organochlorine Pesticides, Polybrominated Diphenyl Ethers, and Perfluoroalkyl Acids in Fish and Blue Crabs of the Lower Passaic River, New Jersey, USA	10.1002/etc.4354

(107)	Trophic behaviors of PFOA and its alternatives perfluoroalkyl ether	10.1016/j.jhazmat.2023.131353
	carboxylic acids (PFECAs) in a coastal food web	
(108)	Disclosing the bioaccumulation and biomagnification behaviors of	10.1016/j.jhazmat.2022.130566
	emerging per/polyfluoroalkyl substances in aquatic food web based	
	on field investigation and model simulation	
(109)	Comprative study of ecodynamic of halogenated micropollutant of	Thesis
	historical and emergent interest in the Seine estuary	
(110)	Bioaccumulation and trophic transfer of perfluorinated alkyl	10.1016/j.envpol.2022.119907
	substances (PFAS) in marine biota from the Belgian North Sea:	
	Distribution and human health risk implications	
(111)	Per-and polyfluoroalkyl-contaminated freshwater impacts adjacent	10.1021/acs.est.0c01640
	riparian food webs	10.1001/ 0.05100
(112)	Quantification of Biodriven Transfer of Per- and Polyfluoroalkyl	10.1021/acs.est.0c0/129
	Substances from the Aquatic to the Terrestrial Environment via	
(110)	Emergent Insects	10.1016/ 10000.00.000
(113)	Perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish	10.1016/j.envpol.2008.03.008
	blood collected near the outfall of wastewater treatment plant	
(114)	(wwiP) in Beljing	10 1020/1-0002021
(114)	investigation of perfluorinated compounds (PFCs) in mollusks from	10.1039/b909302n
(115)	Coastal waters in the Bonal Sea of China Derfluering to d Changing to the Bonal Sea of China	10 1021/0245075
(115)	hetween Experimental and Stable Isotone Dations in Marine	10.1021/es0343973
	Mammals	
(116)	The occurrence tissue distribution and PBT potential of per- and	10 1016/i ibazmat 2023 131868
(110)	polyfluoroalkyl substances in the freshwater organisms from the	10.1010/J.Jnazinat.2025.151000
	Yangtze river via nontarget analysis	
(117)	Bioaccumulation Patterns of Perfluoroalkyl Acids in an Estuary	10 1007/s00128-018-2282-z
(117)	of the Ariake Sea, Japan	101100,,200120 010 2202 2
(9)	Developing methods for assessing trophic magnification of	10.1021/acs.est.3c02361
	perfluoroalkyl substances within an urban terrestrial avian food web	
(118)	Persistent toxic substances in Mediterranean aquatic species	10.1016/j.scitotenv.2014.05.131
(119)	Bioaccumulation and risk mitigation of legacy and novel	10.1016/j.envint.2023.108023
	perfluoroalkyl substances in seafood: Insights from trophic transfer	
	and cooking method	
(120)	Accumulation and exposure assessment of persistent chlorinated and	10.1016/j.scitotenv.2018.07.040
	fluorinated contaminants in Korean birds	
(121)	Levels of chlorinated, brominated, and perfluorinated contaminants	10.7589/2012-03-084
	in birds of prey spanning multiple trophic levels	
(122)	Per-and polyfluoroalkyl substances in waterbird feathers around	10.1016/j.ecoenv.2024.116141
	Poyang Lake, China: Compound and species-specific	
	bioaccumulation	

Supplementary Table 2

Tab. S2. Number of effect sizes (TMF) across food webs in different world regions. Only PFAS with more than 10 effect sizes are reported in the table.

PFAS	Antarctic Region	Arctic	East Asia	West	Europe	Mediterranean Region	North America	South
E 53D	Region	Region		Asia	0		America	America
F-53B	0	0	31	0	0	0	0	0
PFOS	4	4	33	1	19	1	55	0
PFDA	3	2	27	1	18	0	37	0
br-PFOS	0	0	0	0	9	0	0	3
PFUnDA	4	1	32	1	18	0	35	3
I-PFOS	0	0	0	0	15	0	2	3
PFNA	3	2	24	0	15	0	41	3
PFTrDA	1	0	17	0	16	0	29	3
PFDoDA	1	0	28	1	18	0	34	3
FOSA	0	1	2	0	13	0	12	0
PFHxS	1	0	9	1	12	0	20	0
PFDS	0	0	5	0	6	0	11	0
PFTeDA	0	0	6	0	16	0	24	3
PFOA	1	1	23	0	15	0	35	0
PFBA	1	0	14	1	0	0	0	0
PFPeA	0	0	11	0	0	0	0	0
PFHpA	0	0	12	0	3	0	6	0
PFBS	0	0	13	1	0	0	10	0
PFHxA	0	0	13	0	1	0	1	0

Supplementary Table 3

Tab. S3. Summary of international regulation status of selected per- and polyfluoroalkyl substances (PFAS). The table categorises 19 PFAS based on their regulatory status, indicating whether they are listed in the Stockholm Convention, the REACH regulation, or internationally not regulated. A details column provides information on PFAS regulation.

Chemical	Regulation	Details Sources		
PFOS	Stockholm	Listed under the Stockholm Convention	- Stockholm Convention on	
	Convention	on Persistent Organic Pollutants	POPs, Annex B.	
		(POPs). It is also listed in the European	- (123)	
		Drinking Water Directive and Prior	- Environment and Climate	
		Informed Consent (PIC) regulations. It	Change Canada.	
		was banned by the Environment and	- List of New Pollutants for	
		Climate Change Canada (ECCC).	Priority Management	
		Included in the List of New Pollutants	(China).	
		for Priority Management of China.		
PFDA	REACH	Listed in the Registration, Evaluation,	- European Chemicals	
	regulation	Authorisation and Restriction of	Agency (ECHA).	
		Chemicals (REACH) Annex XVII	- REACH regulation.	
		Restricted Substances List (C9-C14	- Thomas et al. (2023).	
		PFCSs). REACH restricts its use and	- Environment and Climate	
		mandates reporting for specific	Change Canada.	
		applications. Also listed in the		
		Classification, Labelling and Packaging		
		(CLP) regulation. It was banned by the		
		ECCC.		
F-53B	Internationally	Developed as a PFOS replacement. It is	- (33)	
	not regulated	not internationally regulated. Currently,		
		F-53B is only used in China. However,		
		it was ubiquitously detected in rivers		
		and lakes in China and the United		
		States, Germany, the United Kingdom,		
		the Netherlands, and South Korea.		
br-PFOS	Stockholm	Considered part of PFOS regulation	- Stockholm Convention on	
	Convention	under the Stockholm Convention.	POPs, Annex B.	

PFUnDA	REACH	Listed in the REACH Annex XVII	- REACH regulation.
	regulation	Restricted C9-C14 PFCSs Substances	- Environment and Climate
		List. It was banned by the ECCC.	Change Canada.
1-PFOS	Stockholm	Considered part of PFOS regulation	- Stockholm Convention on
	Convention	under the Stockholm Convention.	POPs, Annex B.
PFNA	REACH	Listed in the REACH Annex XVII	- ECHA
	regulation	Restricted Substances List (C9-C14	- REACH regulation.
		PFCSs). Also listed in the CLP	- Environment and Climate
		regulation. It was banned by the ECCC.	Change Canada.
PFTrDA	REACH	Listed in the REACH Annex XVII	- REACH regulation.
	regulation	Restricted Substances List (C9-C14	- Environment and Climate
		PFCSs). It was banned by the ECCC.	Change Canada.
PFDoDA	REACH	Listed in the REACH Annex XVII	- REACH regulation.
	regulation	Restricted Substances List (C9-C14	- Environment and Climate
		PFCSs). It was banned by the ECCC.	Change Canada.
FOSA	Stockholm	Regulated as a derivative and	- Stockholm Convention on
	Convention	degradation product of PFOS under the	POPs, Annex B.
		Stockholm Convention, Annex B, as it	
		can degrade to PFOS in the	
		environment.	
PFHxS	Stockholm	Added to the Stockholm Convention in	- Stockholm Convention on
	Convention	2022 under Annex A due to its	POPs, Annex A.
		persistence and potential adverse health	- List of New Pollutants for
		effects. This means PFHxS and its salts	Priority Management
		are targeted for elimination. Listed in	(China).
		the List of New Pollutants for Priority	
		Management of China.	
PFDS	Internationally	Not covered under major international	NA
	not regulated	regulations.	
PFTeDA	REACH	Listed in the REACH Annex XVII	- REACH regulation.
	regulation	Restricted C9-C14 PFCSs Substances	- Environment and Climate
		List. It was banned by the ECCC.	Change Canada.
PFOA	Stockholm	Listed under Annex A of the Stockholm	- Stockholm Convention on
	Convention	Convention with restrictions on	POPs, Annex A.
		production and use, effective since	- Environment and Climate
		2020. Also listed in the CLP and PIC	Change Canada.

		regulations. It was banned by the	- EPA New Zeland.
		ECCC. New Zeland banned Aqueous	- List of New Pollutants for
		Film Forming Foam (AFFF) that	Priority Management
		contain PFOA-related compounds.	(China).
		Listed in the List of New Pollutants for	
		Priority Management of China.	
PFBA	Internationally	Not regulated internationally and not	NA
	not regulated	covered under major regulations.	
PFPeA	Internationally	Not regulated internationally and not	NA
	not regulated	covered under major regulations.	
PFHpA	REACH	Listed as an SVHC under REACH	- REACH Regulation.
	regulation	(group 3). Also listed in the	
		Classification, Labelling and Packaging	
		(CLP) regulation.	
PFBS	REACH	Considered a lower-risk alternative to	- REACH Regulation.
	regulation	PFOS. Listed as an SVHC under	
		REACH (group 2).	
PFHxA	Internationally	Not regulated internationally and not	NA
	not regulated	covered under major regulations.	
		However, it is monitored due to its	
		However, it is monitored due to its increasing use as a replacement for	
		However, it is monitored due to its increasing use as a replacement for long-chain PFAS compounds.	
Tab. S4. Strategies used to handle undetected values. The table presents the types of strategies employed by the studies included in the meta-analysis to address concentrations below the limit of detection or quantification, along with the corresponding number of effect sizes and studies.

Strategy for undetected values	Number of	Number of
	TMFs	Studies
Not provided	337	24
The LOQ value divided by two	81	8
The MDL value divided by two	88	7
The LOD value divided by two	95	5
Zero for values < LOD	24	2
Exclusion of data if values were < LOD	11	1
Exclusion of data if values were < LOQ	96	1
Exclusion of data if values were < MDL.	16	1
Exclusion of values < LOD. The LOQ value divided by two for	7	1
values < LOQ		
Imputation method	31	1
Models accounting for values below the LOD	32	1
Random numbers below half of the MDL	20	1
Random numbers between 0 and the LOD value	36	1
Random numbers between 0 and the LOD value divided by two	24	1
Random numbers between 0 and the MDL value	20	1
The LOD value divided by the square root of two	10	1
The LOD value divided by the square root of two for values < LOD.	7	1
The LOQ value divided by two for values < LOQ		
The LOD value divided by two or detection frequency multiplied by	35	1
LOD value or imputation		
The LOQ value divided by the square root of two	1	1
Three method comparison	33	1
Zero for undetected. The MDL value divided by two for values <	4	1
MDL		
Zero for values < LOD. The LOQ value divided by the square root	12	1
of two for values < LOQ		
Zero for values < LOD. The LOQ value divided by two for values <	10	1
LOQ		

Tab. S5. Deviations and additions to the research protocol. The table outlines the types of deviations and additions to the research protocol, including their descriptions, justifications, the review stage affected, and the magnitude of each deviation.

Deviation /Addition	Description	Reason	Review stage	Impact's
			impacted	magnitude
Changes to the	We removed the	This deviation	Study validity	Low
study validity	performance bias item	was necessary to	assessment	
assessment	and rephrased the	enhance the		
	selection, measurement,	tool's		
	and reporting bias	applicability to		
		the specific types		
		of studies		
		included in the		
		meta-analysis.		
Changes to grey	We replaced the	The OpenGrey	Grey literature	Low
literature search	OpenGrey database with	database was not	search	
databases	the BASE database	working on the		
		day of the		
		search.		
Changes to the	We added the column	The column was	None	NA
TMF_data	"Censored_data_strategy"	added to record		
spreadsheet		the strategy to		
		deal with the		
		undetected data		
Changes to the	We moved the columns	Some studies	None	NA
fw_data spreadsheet	"Sample_type" and	measured TMF		
	"Biomass_conversion"	in the same food		
	from the fw_data	web but using		
	spreadsheet to the	different biomass		
	TMF_data spreadsheet	conversion and		
		or samples		

Tab. S6. PRISMA Eco-Evo checklist. This table presents the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Eco-Evo checklist, tailored for ecological and evolutionary research. It includes key reporting items, corresponding checklist sections, and specific guidelines to ensure transparency, reproducibility, and comprehensiveness in systematic reviews and meta-analyses within eco-evolutionary studies.

Checklist item	Sub-item	Sub-item	Reported	Notes
	number		by authors?	
Title and abstract	1.1	Identify the review as a systematic review,	Yes	Title and
		meta-analysis, or both		abstract
	1.2	Summarise the aims and scope of the	Yes	Abstract
		review		
	1.3	Describe the data set	Yes	Abstract
	1.4	State the results of the primary outcome	Yes	Abstract
	1.5	State conclusions	Yes	Abstract
	1.6	State limitations	No	Limitations
				have their
				own
				paragraph
Aims and questions	2.1	Provide a rationale for the review	Yes	Introduction
	2.2	Reference any previous reviews or meta-	Yes	Introduction
		analyses on the topic		
	2.3	State the aims and scope of the review	Yes	Introduction
		(including its generality)		
	2.4	State the primary questions the review	Yes	Introduction
		addresses (e.g. which moderators were		
		tested)		
	2.5	Describe whether effect sizes were derived	Yes	Introduction
		from experimental and/or observational		
		comparisons		
Review registration	3.1	Register review aims, hypotheses (if	Yes	PROCEED
		applicable), and methods in a time-		(https://doi.
		stamped and publicly accessible archive		<u>org/10.5780</u>
		and provide a link to the registration in the		8/proceed.2
		methods section of the manuscript. Ideally		<u>024.8</u>)
		registration occurs before the search, but it		
		can be done at any stage before data		
		analysis.		

	3.2	Describe deviations from the registered	Yes	Supplement
		aims and methods		ary table 7
	3.3	Justify deviations from the registered aims	Yes	Supplement
		and methods		ary table 7
Eligibility criteria	4.1	Report the specific criteria used for	Yes	Supplement
		including or excluding studies when		ary method
		screening titles and/or abstracts, and full		1 and 2
		texts, according to the aims of the		
		systematic review (e.g. study design, taxa,		
		data availability)		
	4.2	Justify criteria, if necessary (i.e. not	Yes	Supplement
		obvious from aims and scope)		ary method
				1 and 2
Finding studies	5.1	Define the type of search (e.g.	Yes	Supplement
		comprehensive search, representative		ary method
		sample)		1
	5.2	State what sources of information were	Yes	Supplement
		sought (e.g. published and unpublished		ary method
		studies, personal communications)		1
	5.3	Include, for each database searched, the	Yes	Supplement
		exact search strings used, with keyword		ary table 10
		combinations and Boolean operators		
	5.4	Provide enough information to repeat the	Yes	Supplement
		equivalent search (if possible), including		ary table 7
		the timespan covered (start and end dates)		
Study selection	6.1	Describe how studies were selected for	Yes	Materials
		inclusion at each stage of the screening		and
		process (e.g. use of decision trees,		Methods
		screening software)		
	6.2	Report the number of people involved and	Yes	Materials
		how they contributed (e.g. independent		and
		parallel screening)		Methods
Data collection	7.1	Describe where in the reports data were	Yes	Supplement
process		collected from (e.g. text or figures)		ary Data
	7.2	Describe how data were collected (e.g.	Yes	NA
		software used to digitize figures, external		
		data sources)		
	7.3	Describe moderator variables that were	Yes	Table 1
		constructed from collected data (e.g.		

		number of generations calculated from		
		years and average generation time)		
	7.4	Report how missing or ambiguous	Yes	Materials
		information was dealt with during data		and
		collection (e.g. authors of original studies		Methods
		were contacted for missing descriptive		
		statistics, and/or effect sizes were		
		calculated from test statistics)		
	7.5	Report who collected data	Yes	Materials
				and
				Methods
	7.6	State the number of extractions that were	No	NA
		checked for accuracy by co-authors		
Data items	8.1	Describe the key data sought from each	Yes	Pre-
		study		registered
				protocol
	8.2	Describe items that do not appear in the	No	NA
		main results, or which could not be		
		extracted due to insufficient information		
	8.3	Describe main assumptions or	Yes	Discussion
		simplifications that were made (e.g.		
		categorising both 'length' and 'mass' as		
		'morphology')		
	8.4	Describe the type of replication unit (e.g.	Yes	Materials
		individuals, broods, study sites)		and
				Methods
Assessment of	9.1	Describe whether the quality of studies	Materials	Sensitivity
individual study		included in the systematic review or meta-	and Methods	analysis
quality		analysis was assessed (e.g. blinded data		
		collection, reporting quality, experimental		
		versus observational)		
	9.2	Describe how information about study	Materials	Sensitivity
		quality was incorporated into analyses (e.g.	and Methods	analysis
		meta-regression and/or sensitivity analysis)		
Effect size	10.1	Describe effect size(s) used	Yes	Materials
measures				and
				Methods

	10.2	Provide a reference to the equation of each	Yes	Materials
		calculated effect size (e.g. standardised		and
		mean difference, log response ratio) and (if		Methods
		applicable) its sampling variance		
	10.3	If no reference exists, derive the equations	Yes	Materials
		for each effect size and state the assumed		and
		sampling distribution(s)		Methods
Missing data	11.1	Describe any steps taken to deal with	Yes	Materials
		missing data during analysis (e.g.		and
		imputation, complete case, subset analysis)		Methods &
				Supplement
				ary
				Methods
	11.2	Justify the decisions made to deal with	Yes	Materials
		missing data		and
		-		Methods &
				Supplement
				ary
				Methods
Meta-analytic	12.1	Describe the models used for synthesis of	Yes	Materials
model description		effect sizes		and
				Methods
	12.2	The most common approach in ecology	Yes	Materials
		and evolution will be a random-effects		and
		model, often with a hierarchical/multilevel		Methods
		structure. If other types of models are		
		chosen (e.g. common/fixed effects model,		
		unweighted model), provide justification		
		for this choice		
Software	13.1	Describe the statistical platform used for	Yes	Materials
		inference (e.g. <i>R</i>)		and
				Methods
	13.2	Describe the packages used to run models	Yes	Materials
				and
				Methods
	13.3	Describe the functions used to run models	Yes	Materials
				and
				Methods

	13.4	Describe any arguments that differed from	Yes	Materials
		the default settings		and
				Methods
	13.5	Describe the version numbers of all	Yes	NA
		software used		
Non-independence	14.1	Describe the types of non-independence	Yes	Materials
		encountered (e.g. phylogenetic, spatial,		and
		multiple measurements over time)		Methods
	14.2	Describe how non-independence has been	Yes	Materials
		handled		and
				Methods
	14.3	Justify decisions made	Yes	Materials
				and
				Methods
Meta-regression	15.1	Provide a rationale for the inclusion of	Yes	Table 1
and model		moderators (covariates) that were		
selection		evaluated in meta-regression models		
	15.2	Justify the number of parameters estimated	Yes	Materials
		in models, in relation to the number of		and
		effect sizes and studies (e.g. interaction		Methods
		terms were not included due to insufficient		
		sample sizes)		
	15.3	Describe any process of model selection	Yes	Materials
				and
				Methods
Publication bias	16.1	Describe assessments of the risk of bias	Yes	Publication
and sensitivity		due to missing results (e.g. publication,		bias section
analyses		time-lag, and taxonomic biases)		
	16.2	Describe any steps taken to investigate the	Yes	Publication
		effects of such biases (if present)		bias section
	16.3	Describe any other analyses of robustness	Yes	Publication
		of the results, e.g. due to effect size choice,		bias and
		weighting or analytical model		sensitivity
		assumptions, inclusion or exclusion of		analyses
		subsets of the data, or the inclusion of		
		alternative moderator variables in meta-		
		regressions		
Clarification of	17.1	When hypotheses were formulated after	Yes	Table 1
post hoc analyses		data analysis, this should be		
		acknowledged.		

Metadata, data, and	18.1	Share metadata (i.e. data descriptions)	Yes	Pre-
code				registered
				protocol
				and
				Supplement
				ary Data
	18.2	Share data required to reproduce the	Yes	Supplement
		results presented in the manuscript		ary Data
	18.3	Share additional data, including	Yes	Supplement
		information that was not presented in the		ary Data
		manuscript (e.g. raw data used to calculate		
		effect sizes, descriptions of where data		
		were located in papers)		
	18.4	Share analysis scripts (or, if a software	Yes	GitHub
		package with graphical user interface		Repository
		(GUI) was used, then describe full model		
		specification and fully specify choices)		
Results of study	19.1	Report the number of studies screened	Yes	Results
selection process	19.2	Report the number of studies excluded at	Yes	Results
		each stage of screening		
	19.3	Report brief reasons for exclusion from the	Yes	Supplement
		full text stage		sry Table 2
	19.4	Present a Preferred Reporting Items for	Yes	Supplement
		Systematic Reviews and Meta-Analyses		ary Figure 1
		(PRISMA)-like flowchart (www.prisma-		
		statement.org).		
Sample sizes and	20.1	Report the number of studies and effect	Yes	Results
study		sizes for data included in meta-analyses		
characteristics	20.2	Report the number of studies and effect	Yes	Results
		sizes for subsets of data included in meta-		
		regressions		
	20.3	Provide a summary of key characteristics	Yes	Results
		for reported outcomes (either in text or		
		figures; e.g. one quarter of effect sizes		
		reported for vertebrates and the rest		
		invertebrates)		

	20.4	Provide a summary of limitations of	Yes	Results
		included moderators (e.g. collinearity and		
		overlap between moderators)		
	20.5	Provide a summary of characteristics	Yes	Results
		related to individual study quality (risk of		
		bias)		
Meta-analysis	21.1	Provide a quantitative synthesis of results	Yes	Results
		across studies, including estimates for the		
		mean effect size, with confidence/credible		
		intervals		
Heterogeneity	22.1	Report indicators of heterogeneity in the	Yes	Results
		estimated effect (e.g. I^2 , tau^2 and other		
		variance components)		
Meta-regression	23.1	Provide estimates of meta-regression	Yes	Results
		slopes (i.e. regression coefficients) and		
		confidence/credible intervals		
	23.2	Include estimates and confidence/credible	Yes	Results
		intervals for all moderator variables that		
		were assessed (i.e. complete reporting)		
	23.3	Report interactions, if they were included	Yes	Results
	23.4	Describe outcomes from model selection,	Yes	Results
		if done (e.g. R2 and AIC)		
Outcomes of	24.1	Provide results for the assessments of the	Yes	Results
publication bias		risks of bias (e.g. Egger's regression,		
and sensitivity		funnel plots)		
analyses	24.2	Provide results for the robustness of the	Yes	Results
		review's results (e.g. subgroup analyses,		
		meta-regression of study quality, results		
		from alternative methods of analysis, and		
		temporal trends)		
Discussion	25.1	Summarise the main findings in terms of	Yes	Discussion
		the magnitude of effect		
	25.2	Summarise the main findings in terms of	Yes	Discussion
		the precision of effects (e.g. size of		
		confidence intervals, statistical		
		significance)		

	25.3	Summarise the main findings in terms of	Yes	Discussion
		their heterogeneity		
	25.4	Summarise the main findings in terms of	Yes	Discussion
		their biological/practical relevance		
	25.5	Compare results with previous reviews on	Yes	Discussion
		the topic, if available		
	25.6	Consider limitations and their influence on	Yes	Discussion
		the generality of conclusions, such as gaps		
		in the available evidence (e.g. taxonomic		
		and geographical research biases)		
Contributions and	26.1	Provide names, affiliations, and funding	Yes	NA
funding		sources of all co-authors		
	26.2	List the contributions of each co-author	Yes	NA
	26.3	Provide contact details for the	Yes	NA
		corresponding author		
	26.4	Disclose any conflicts of interest	Yes	NA
References	27.1	Provide a reference list of all studies	Yes	NA
		included in the systematic review or meta-		
		analysis		
	27.2	List included studies as referenced sources	Yes	NA
		(e.g. rather than listing them in a table or		
		supplement)		

Tab. S7. Digital Object Identifiers (DOIs) of the 25 banchmark bibliographic records. These records were specifically selected to validate the thoroughness of the search methodology, evaluating our search strategy's sensitivity and comprehensiveness.

DOIs:
10.1021/es049331s
10.1021/es049620g
10.1021/es060233b
10.1021/es800906r
10.1021/es9003894
10.1021/es900162n
10.1021/es200432n
10.1016/j.envpol.2013.09.011
10.1021/es405018b
10.1021/es504445x
10.1021/acs.est.7b02399
10.1016/j.envpol.2018.05.087
10.1016/j.envpol.2018.05.001
10.1039/C9EM00322C
10.1016/j.scitotenv.2019.05.461
10.1021/acs.est.9b05007
10.1016/j.envpol.2019.113383
10.3390/w12061591
10.1021/acs.est.0c04587
10.1016/j.envint.2020.105621
10.1016/j.scitotenv.2020.142146
10.1016/j.jhazmat.2020.124681
10.1021/acs.est.1c00965
10.1016/j.jhazmat.2020.123618
10.1016/j.jglr.2021.08.013
Danahurant ayamu

Benchmark query:

DOI(10.1021/es049331s) OR DOI(10.1021/es049620g) OR DOI(10.1021/es060233b) OR DOI(10.1021/es800906r) OR DOI(10.1021/es9003894) OR DOI(10.1021/es900162n) OR DOI(10.1021/es200432n) OR DOI(10.1016/j.envpol.2013.09.011) OR DOI(10.1021/es405018b) OR DOI(10.1021/es504445x) OR DOI(10.1021/acs.est.7b02399) OR DOI(10.1016/j.envpol.2018.05.087) OR DOI(10.1016/j.envpol.2018.05.001) OR DOI(10.1039/C9EM00322C) OR DOI(10.1016/j.scitotenv.2019.05.461) OR DOI(10.1021/acs.est.9b05007) OR DOI(10.1016/j.envpol.2019.113383) OR DOI(10.3390/w12061591) OR DOI(10.1021/acs.est.0c04587) OR DOI(10.1016/j.envint.2020.105621) OR DOI(10.1016/j.scitotenv.2020.142146) OR DOI(10.1016/j.jhazmat.2020.124681) OR DOI(10.1021/acs.est.1c00965) OR DOI(10.1016/j.jhazmat.2020.123618) OR DOI(10.1016/j.jglr.2021.08.013)

Tab. S8. Searches records from six online databases. This table provides details of the systematic searches conducted across six online databases. It includes the database names, search strings tailored to each database's structure, the dates of the searches, and the number of resulting records (hits). The search strings were designed to be comprehensive, aiming to capture all relevant studies on *PFAS* concentrations within food webs.

Source	Search string	Date of	Number
		search	of hits
		0.0 /0.4 /0.00.4	
PubMed	("fluoroalkyl" OR perfluo* OR polyfluo* OR organofluorine OR pfas	08/04/2024	526
	OR pfass OR pfba OR pfpea OR pfhxa OR pfhpa OR pfoa OR pfna OR		
	pfda OR pfdea OR pfdca OR pfunda OR pfuna OR pfua OR pfuda OR		
	pfdoa OR pfdoda OR pftrda OR pftrida OR pftra OR pfta OR pfteda OR		
	pfo4da OR pfo5doda OR pfbs OR pfbus OR pfpes OR pfhxs OR pfhps		
	OR pfos OR pfns OR pfds OR fts OR ftsa OR pfechs OR fosa OR pfosa		
	OR netfosaa OR "Et-PFOSA-AcOH" OR nmefosaa OR "Me-PFOSA-		
	AcOH" OR adona OR "CI-PFAES" OR "F-53B" OR genx OR "HFPO-		
	TA" OR "Hydro-Eve" OR "Nafion BP2" OR teflon OR tefal OR c8 OR		
	"emerging pollutant*" OR organohalogen* OR ptfe OR fluorotelomer*)		
	AND ("trophic level*" OR "trophic position*" OR tropho* OR "trophic		
	amplification" OR "trophic magnification*" OR "trophic transfer" OR		
	bioamplification* OR biomagnif* OR "biological magnification*"OR		
	"cumulative concentration*" OR "food chain*" OR "food web*" OR		
	TMF OR "magnification factor*") NOT review[pt]		
Web of Science	TS=("fluoroalkyl*" OR perfluo* OR polyfluo* OR organofluorine OR	08/04/2024	949
(Core	pfas OR pfass OR pfba OR pfpea OR pfhxa OR pfhpa OR pfoa OR pfna		
Collection)	OR pfda OR pfdea OR pfdca OR pfunda OR pfuna OR pfua OR pfuda		
	OR pfdoa OR pfdoda OR pftrda OR pftrida OR pftra OR pfta OR pfteda		
	OR pfo4da OR pfo5doda OR pfbs OR pfbus OR pfpes OR pfhxs OR		
	pfhps OR pfos OR pfns OR pfds OR fts OR ftsa OR pfechs OR fosa OR		
	pfosa OR netfosaa OR "Et-PFOSA-AcOH" OR nmefosaa OR "Me-		
	PFOSA-AcOH" OR adona OR "Cl-PFAES" OR "F-53B" OR genx OR		
	"HFPO-TA" OR "Hydro-Eve" OR "Nafion BP2" OR teflon OR tefal OR		
	c8 OR "emerging pollutant*" OR organohalogen* OR ptfe OR		
	fluorotelomer*) AND TS=("nitrogen isotope*" OR "stable isotope*" OR		
	"trophic level*" OR "trophic position*" OR tropho* OR "trophic		
	amplification" OR "trophic magnification*" OR "trophic transfer" OR		
	bioamplification* OR biomagnif* OR "biological magnification*" OR		

	"aumulative concentration*" OD "food shein*" OD "food web*" OD		
	TME OD "me arification for tast") NOT DT=(D minut)		
	TMF OK magnification factor.) NOT DT=(Review)		
Scopus	TITLE-ABS-KEY (*fluoroalkyl* OR perfluo* OR polyfluo*	08/04/2024	1543
	organofluorine OR pfas OR pfass OR pfba OR pfpea OR pfhxa OR		
	pfhpa OR pfoa OR pfna OR pfda OR pfdea OR pfdca OR pfunda OR		
	pfuna OR pfua OR pfuda OR pfdoa OR pfdoda OR pftrda OR pftrida		
	OR pftra OR pfta OR pfteda OR pfo4da OR pfo5doda OR pfbs OR		
	pfbus OR pfpes OR pfhxs OR pfhps OR pfos OR pfns OR pfds OR fts		
	OR ftsa OR pfechs OR fosa OR pfosa OR netfosaa OR "Et-PFOSA-		
	AcOH" OR nmefosaa OR "Me-PFOSA-AcOH" OR adona OR "Cl-		
	PFAES" OR "F-53B" OR genx OR "HFPO-TA" OR "Hydro-Eve" OR		
	"Nafion BP2" OR teflon OR tefal OR c8 OR "emerging pollutant*" OR		
	organohalogen* OR ptfe OR fluorotelomer*) AND ("trophic level*"		
	OR "trophic position*" OR "trophic amplification" OR "trophic		
	magnification*" OR "trophic transfer" OR bioamplification* OR		
	biomagnif* OR "biological magnification*" OR "magnification factor*"		
	OR TMF) AND (EXCLUDE (DOCTYPE, "re"))		
GreenFile	(fluoroalkyl OR perfluo* OR polyfluo* OR organofluorine OR pfas OR	08/04/2024	392
(EBSCO)	pfass OR pfba OR pfpea OR pfhxa OR pfhpa OR pfoa OR pfna OR pfda		
	OR pfdea OR pfdca OR pfunda OR pfuna OR pfua OR pfuda OR pfdoa		
	OR pfdoda OR pftrda OR pftrida OR pftra OR pfta OR pfteda OR		
	pfo4da OR pfo5doda OR pfbs OR pfbus OR pfpes OR pfhxs OR pfhps		
	OR pfos OR pfns OR pfds OR fts OR ftsa OR pfechs OR fosa OR pfosa		
	OR netfosaa OR "Et-PFOSA-AcOH" OR nmefosaa OR "Me-PFOSA-		
	AcOH" OR adona OR "CI-PFAES" OR "F-53B" OR genx OR "HFPO-		
	TA" OR "Hydro-Eve" OR "Nafion BP2" OR teflon OR tefal OR c8 OR		
	"emerging pollutant*" OR organohalogen* OR ptfe OR fluorotelomer*)		
	AND (nitrogen isotope* OR stable isotope* OR trophic level* OR		
	"trophic position*" OR tropho* OR "trophic amplification" OR "trophic		
	magnification*" OR "trophic transfer" OR bioamplification* OR		
	biomagnif* OR "biological magnification*" OR "cumulative		
	concentration*" OR "food chain*" OR "food web*" OR "magnification		
	factor*" OR TMF)		
BASE	(PFAS* OR *fluoroalkyl* OR PFOS OR PFOA) AND (trophic	08/04/2024	89
(Bielefeld	magnification OR tmf OR biomagnification) doctype:(18* 19)		
Academic			
Search Engine)			

ProQuest -	(noft("perfluoroalky1*") OR noft("polyfluoroalky1*") OR noft(pfas) OR	08/04/2024	313	
Theses and	noft(pfos) noft(pfoa)) AND (noft("biomagnification") OR noft("trophic			
Dissertations	magnification") OR noft("magnification factor") noft("food web")			
Database	noft("TMF")) NOT noft(DocumentType:Review)NOT			
	noft(DocumentType:Review)			
Dissertations Database	magnification") OR noft("magnification factor") noft("food web") noft("TMF")) NOT noft(DocumentType:Review)NOT noft(DocumentType:Review)			

Tab. S9. Excluded studies at the full-text screening stage. This table provides detailed information on studies excluded from the metaanalysis following the full-text screening, along with specific reasons for their exclusion.

Title	Year	DOI	Exclusion reason
Per-and poly-fluoroalkyl compounds	2017	10.1016/j.scitotenv.2017.06.111	The necessary data to
in freshwater fish from the			calculate TMF and standard
Rhcircumflex [~] ne River: influence of			error were not provided.
fish size, diet, prey contamination			
and biotransformation.			
Identification, Tissue Distribution,	2016	10.1021/acs.est.6b01980	The necessary data to
and Bioaccumulation Potential of			calculate TMF and standard
Cyclic Perfluorinated Sulfonic Acids			error were not provided.
Isomers in an Airport Impacted			
Ecosystem			
Bioaccumulation of Fluorotelomer	NA	10.1021/acs.est.9b00927	The necessary data to
Sulfonates and Perfluoroalkyl Acids			calculate TMF and standard
in Marine Organisms Living in			error were not provided.
Aqueous Film-Forming Foam			
Impacted Waters			
The driving factors of per- and	NA	10.1016/j.scitotenv.2021.151662	The necessary data to
polyfluorinated alkyl substance			calculate TMF and standard
(PFAS) accumulation in selected fish			error were not provided.
species: The influence of position in			
river continuum, fish feed			
composition, and pollutant properties			
Bioaccumulation of emerging	2016	10.1016/j.envres.2016.05.004	The necessary data to
organic compounds (perfluoroalkyl			calculate TMF and standard
substances and halogenated flame			error were not provided.
retardants) by earthworm in biosolid			
amended soils			
Perfluorinated compounds in aquatic	NA	10.1007/s00244-004-0133-x	The necessary data to
organisms at various trophic levels in			calculate TMF and standard
a Great Lakes food chain			error were not provided.
Polyfluorinated chemicals in a	NA	10.1016/j.chemosphere.2007.06.067	The necessary data to
spatially and temporally integrated			calculate TMF and standard
food web in the Western Arctic			error were not provided.

The impact of precursors on aquatic	NA	10.1002/ieam.4414	The necessary data to
exposure assessment for PFAS:			calculate TMF and standard
Insights from bioaccumulation			error were not provided.
modeling			
A nationwide survey of	NA	10.1016/j.jhazmat.2016.04.010	The necessary data to
perfluorinated alkyl substances in			calculate TMF and standard
waters, sediment and biota collected			error were not provided.
from aquatic environment in			
Vietnam: Distributions and			
bioconcentration profiles			
Bioaccumulation and effects of novel	2018	10.1016/j.envpol.2017.10.039	The necessary data to
chlorinated polyfluorinated ether			calculate TMF and standard
sulfonate in freshwater alga			error were not provided.
<i>Scenedesmus obliquus</i>			
Persistent organic pollutants in lakes	NA	10.1007/s10646-019-02045-x	No PFAS.
of Broknes peninsula at Larsemann			
Hills area, East Antarctica			
Perfluorinated Chemicals in	2012	NA	The necessary data to
Meromictic Lakes on the Northern			calculate TMF and standard
Coast of Ellesmere Island, High			error were not provided.
Arctic Canada			
Bioaccumulation and metabolic	2022	10.1016/j.scitotenv.2021.151264	The necessary data to
response of PFAS mixtures in wild-			calculate TMF and standard
caught freshwater turtles (Emydura			error were not provided.
macquarii macquarii) using omics-			
based ecosurveillance techniques			
Perfluorinated contaminants in	NA	10.1021/es0603195	The necessary data to
sediments and aquatic organisms			calculate TMF and standard
collected from shallow water and			error were not provided.
tidal flat areas of the Ariake Sea,			
Japan: environmental fate of			
perfluorooctane sulfonate in aquatic			
ecosystems			
Characterisation of PFASs and	2020	NA	Duplicate data
Organofluorine in Freshwater			
Environments : Transfer from water			
to land via emergent aquatic insects			
Levels, Patterns, and	NA	10.1021/acs.est.9b02533	The necessary data to
Biomagnification Potential of			calculate TMF and standard

Terrestrial Food Chain in a Nordic			
Skiing Area			
Bioaccumulation and	NA	10.1016/j.envpol.2019.06.035	The necessary data to
biomagnification of perfluoroalkyl			calculate TMF and standard
acids and precursors in East			error were not provided.
Greenland polar bears and their			
ringed seal prey			
Bioaccumulation of Perfluoroalkyl	2024	10.1021/acs.estlett.4c00143	The necessary data to
Sulfonamides (FASA)			calculate TMF and standard
			error were not provided.
Persistent organic pollutants in biotic	2023	NA	The necessary data to
and abiotic components of the			calculate TMF and standard
Orange-Senqu River basin			error were not provided.
Pollution Characteristics of	2019	10.13227/j.hjkx.201901104	Full-text not available.
Perfluorinated Alkyl Substances			
(PFASs) in Seawater, Sediments, and			
Biological Samples from Jiaozhou			
Bay, China			
Perfluoroalkyl acids and	NA	10.1016/j.envres.2020.110151	Only one species was
sulfonamides and dietary, biological			investigated.
and ecological associations in			
peregrine falcons from the			
Laurentian Great Lakes Basin,			
Canada			
Levels and profiles of perfluorinated	NA	10.1016/j.scitotenv.2021.151263	The necessary data to
alkyl acids in liver tissues of birds			calculate TMF and standard
with different habitat types and			error were not provided.
trophic levels from an urbanized			
coastal region of South Korea			
Bioaccumulation characteristics of	2015	10.1016/j.chemosphere.2014.06.023	The necessary data to
perfluoroalkyl acids (PFAAs) in			calculate TMF and standard
coastal organisms from the west			error were not provided.
coast of South Korea			
Dietary bioaccumulation of	2020	10.1016/j.ecolmodel.2020.109196	The necessary data to
persistent organic pollutants in the			calculate TMF and standard
common sole <i>Solea solea</i> in			error were not provided.
the context of global change. Part 2:			
Sensitivity of juvenile growth and			
contamination to toxicokinetic			
parameters uncertainty and			

environmental conditions variability			
in estuaries			
Perfluoroalkyl acids in various edible	2015	10.1016/j.chemosphere.2014.08.077	The necessary data to
Baltic, freshwater, and farmed fish in			calculate TMF and standard
Finland			error were not provided.
Direct evidence of the important role	NA	10.1016/j.scitotenv.2022.161012	The necessary data to
of proteins in bioconcentration and			calculate TMF and standard
biomagnification of PFASs in			error were not provided.
benthic organisms based on			
comparison with OPEs			
Dietary bioaccumulation of	NA	10.1021/es204533m	The necessary data to
perfluorophosphonates and			calculate TMF and standard
perfluorophosphinates in juvenile			error were not provided.
rainbow trout: evidence of			
metabolism of perfluorophosphinates			
A food web bioaccumulation model	NA	10.1039/d2em00047d	The necessary data to
for the accumulation of per- and			calculate TMF and standard
polyfluoroalkyl substances (PFAS) in			error were not provided.
fish: how important is renal			
elimination?			
PFAS and Precursor	NA	10.1021/acs.est.2c03734	The necessary data to
Bioaccumulation in Freshwater			calculate TMF and standard
Recreational Fish: Implications for			error were not provided.
Fish Advisories			
Bioaccumulation of perfluoroalkyl	2013	10.1016/j.envpol.2013.04.002	The necessary data to
carboxylates (PFCAs) and			calculate TMF and standard
perfluoroalkane sulfonates (PFSAs)			error were not provided.
by earthworms (<i>Eisenia</i>			
fetida) in soil			
Bioaccumulation of perfluoroalkyl	2018	10.1016/j.marpolbul.2018.04.029	The necessary data to
substances in exploited fish and			calculate TMF and standard
crustaceans: Spatial trends across			error were not provided.
two estuarine systems			
Per- and polyfluoroalkyl substances	2021	NA	The necessary data to
(PFAS) in ski products:			calculate TMF and standard
Environmental contamination,			error were not provided.
bioaccumulation and effects in			
rodents			

Nonlethal detection of PFAS	NA	10.1016/j.envpol.2023.121123	The necessary data to
bioaccumulation and			calculate TMF and standard
biomagnification within fishes in an			error were not provided.
urban- and wastewater-dominant			
Great Lakes watershed			
Environmental fate of poly- and	NA	NA	Duplicate data
perfluoroalkyl substances (PFAS) in			
aquatic systems : identification of			
urban sources and trophic transfer			
assessment ; Ecodynamique des			
substances poly- et			
perfluoroalkyl \tilde{A} ©es (PFAS) dans les			
syst \tilde{A} ["] mes aquatiques : identification			
des sources en milieu urbain et			
évalu			
Field-Based Distribution and	2022	10.1021/acs.est.2c01965	The necessary data to
Bioaccumulation Factors for Cyclic			calculate TMF and standard
and Aliphatic Per- and			error were not provided.
Polyfluoroalkyl Substances (PFASs)			
in an Urban Sedentary Waterbird			
Population			
Understanding PFAAs exposure in a	2021	10.1016/j.envpol.2020.116355	Only one species
generalist seabird species breeding in			investigated.
the vicinity of a fluorochemical			
plant: Influence of maternal transfer			
and diet			
Bioaccumulation of perfluoroalkyl	2019	10.1016/j.chemosphere.2018.10.037	The necessary data to
substances in marine echinoderms:			calculate TMF and standard
Results of laboratory-scale			error were not provided.
experiments with <i>Holothuria</i>			
tubulosa Gmelin, 1791			
Effect of abiotic factors and	NA	10.1016/j.scitotenv.2021.149448	The necessary data to
environmental concentrations on the			calculate TMF and standard
bioaccumulation of persistent organic			error were not provided.
and inorganic compounds to			
freshwater fish and mussels			
Distribution of	2021	NA	The necessary data to
perfluorooctanesulfonate (PFOS)			calculate TMF and standard
isomers in a Norwegian arctic food			error were not provided.
web			

Perfluorinated compounds in surface	NA	10.1007/s00128-012-0745-1	The necessary data to
water and organisms from			calculate TMF and standard
Baiyangdian Lake in North China:			error were not provided.
source profiles, bioaccumulation and			
potential risk			
First insights into per-and	NA	10.1016/j.chemosphere.2023.140970	The necessary data to
polyfluoroalkyl substance			calculate TMF and standard
contamination in edible fish species			error were not provided.
of the Indus water system of Pakistan			
Assessment of metal and organic	NA	10.1016/j.marenvres.2024.106432	No PFAS.
pollutants in combination with stable			
isotope analysis in tunas from the			
Gulf of Cadiz (east Atlantic)			
Bioaccumulation and risk assessment	2014	10.1016/j.ecoenv.2014.05.031	The necessary data to
of per- and polyfluoroalkyl			calculate TMF and standard
substances in wild freshwater fish			error were not provided.
from rivers in the Pearl River Delta			
region, South China			
A survey of perfluorinated	NA	10.1016/j.chemosphere.2009.02.055	The necessary data to
compounds in surface water and			calculate TMF and standard
biota including dolphins from the			error were not provided.
Ganges River and in other			
waterbodies in India			
The relationship between	NA	10.1016/j.scitotenv.2009.07.032	The necessary data to
perfluorinated chemical levels in the			calculate TMF and standard
feathers and livers of birds from			error were not provided.
different trophic levels			
Tissue distribution and	2021	10.1016/j.envpol.2020.115887	The necessary data to
bioaccumulation of legacy and			calculate TMF and standard
emerging per- and polyfluoroalkyl			error were not provided.
substances (PFASs) in edible fishes			
from Taihu Lake, China			
Survey of legacy and emerging per-	NA	10.1016/j.envpol.2021.118398	The necessary data to
and polyfluorinated alkyl substances			calculate TMF and standard
in Mediterranean seafood from a			error were not provided.
North African ecosystem			PFAS concentrations pooled
			in major taxa and not
			provided at the species
			level.

Perfluorinated alkyl substances	2015	10.1016/j.chemosphere.2014.11.044	The necessary data to
(PFAS) in terrestrial environments in			calculate TMF and standard
Greenland and Faroe Islands			error were not provided.
STUDIO DELLA	2022	NA	The necessary data to
BIODISTRIBUZIONE DI			calculate TMF and standard
SOSTANZE BIOLOGICAMENTE			error were not provided.
ATTIVE IN ORGANISMI			
ACQUATICI			
Biomagnification and temporal	NA	10.1016/j.envpol.2023.122738	The necessary data to
trends (1990-2021) of perfluoroalkyl			calculate TMF and standard
substances in striped dolphins			error were not provided.
(Stenella coeruleoalba) from the NW			
Mediterranean sea			
Characterisation of perfluorooctane	NA	10.1007/s11356-013-2449-4	The necessary data to
sulfonate (PFOS) in a terrestrial			calculate TMF and standard
ecosystem near a fluorochemical			error were not provided.
plant in Flanders, Belgium			
The fate of poly- and perfluoroalkyl	NA	10.1039/d0em00510j	The necessary data to
substances in a marine food web			calculate TMF and standard
influenced by land-based sources in			error were not provided.
the Norwegian Arctic			
Enantiospecific perfluorooctane	NA	10.1021/es301160r	The necessary data to
sulfonate (PFOS) analysis reveals			calculate TMF and standard
evidence for the source contribution			error were not provided.
of PFOS-precursors to the Lake			
Ontario foodweb			
Fractionation of perfluoroalkyl acids	NA	10.1016/j.chemosphere.2023.137931	The necessary data to
(PFAAs) along the aquatic food			calculate TMF and standard
chain promoted by competitive			error were not provided.
effects between longer and shorter			
chain PFAAs			
Accumulation of per- and	2023	10.1101/2023.12.12.571392	The necessary data to
polyfluoroalkyl substances (PFAS) in			calculate TMF and standard
a terrestrial food web			error were not provided.
Transfer of perfluorinated	2014	NA	The necessary data to
compounds from sediment to benthic			calculate TMF and standard
invertebrates and fish			error were not provided.
Bioaccumulation of	NA	10.1016/j.envres.2014.12.022	no PFAS.
organohalogenated compounds in			

sharks and rays from the			
southeastern USA			
Dietary accumulation of	2003	10.1002/etc.5620220125	The necessary data to
perfluorinated acids in juvenile			calculate TMF and standard
rainbow trout (Oncorhynchus			error were not provided.
mykiss)			
Perfluorinated compounds in the	NA	10.1016/j.scitotenv.2019.135047	The necessary data to
aquatic food chains of two			calculate TMF and standard
subtropical estuaries			error were not provided.
Perfluorinated alkyl substances in	2014	10.1016/j.scitotenv.2014.01.045	The necessary data to
water, sediment, plankton and fish			calculate TMF and standard
from Korean rivers and lakes: A			error were not provided.
nationwide survey			
Detection of a Cyclic Perfluorinated	2011	10.1021/es200135c	The necessary data to
Acid, Perfluoroethylcyclohexane			calculate TMF and standard
Sulfonate, in the Great Lakes of			error were not provided.
North America			
Dietary exposure and accumulation	NA	10.1016/j.scitotenv.2020.142730	The necessary data to
of per- and polyfluoroalkyl			calculate TMF and standard
substances alters growth and reduces			error were not provided.
body condition of post-metamorphic			
salamanders			
Ecosystem specific accumulation of	NA	10.1016/j.envres.2022.113455	The necessary data to
organohalogenated compounds: A			calculate TMF and standard
comparison between adjacent			error were not provided.
freshwater and terrestrial avian			
predators			
Perfluorinated compounds in the	2006	10.1007/698_5_046	Review.
Great Lakes			
Identification of long-chain	NA	10.1021/es034727+	The necessary data to
perfluorinated acids in biota from the			calculate TMF and standard
Canadian Arctic			error were not provided.
Legacy and emerging	NA	10.1016/j.scitotenv.2017.10.296	The necessary data to
organohalogenated contaminants in			calculate TMF and standard
wild edible aquatic organisms:			error were not provided.
Implications for bioaccumulation and			
human exposure			
Trophic transfer of PFAS from	2022	10.1016/j.envpol.2022.119814	The necessary data to
tomato (Solanum lycopersicum) to			calculate TMF and standard
			error were not provided.

tobacco hornworm (Manduca sexta)			
caterpillars			
Accumulation of perfluorooctane	NA	10.1016/j.chemosphere.2007.08.038	The necessary data to
sulfonate (PFOS) in the food chain of			calculate TMF and standard
the Western Scheldt estuary:			error were not provided.
Comparing field measurements with			
kinetic modeling			
Perfluoroalkyl substances in	2022	10.1016/j.marpolbul.2022.113995	The necessary data to
freshwater and marine fish from			calculate TMF and standard
northern Vietnam: Accumulation			error were not provided.
levels, profiles, and implications for			
human consumption			
Perfluoroalkyl substances in the	NA	10.1007/s11356-022-20753-6	The necessary data to
surface water and fishes in Chaohu			calculate TMF and standard
Lake, China			error were not provided.
Elevated levels of per- and	2022	10.1016/j.chemosphere.2021.132830	The necessary data to
polyfluoroalkyl substances (PFAS) in			calculate TMF and standard
freshwater benthic			error were not provided.
macroinvertebrates from the Hudson			
River Watershed			
Spatial (bio)accumulation of	NA	10.1016/j.envpol.2017.11.090	The necessary data to
pharmaceuticals, illicit drugs,			calculate TMF and standard
plasticisers, perfluorinated			error were not provided.
compounds and metabolites in river			
sediment, aquatic plants and benthic			
organisms			
Perfluorinated compounds: levels,	NA	10.1016/j.marpolbul.2013.09.014	The necessary data to
trophic web enrichments and human			calculate TMF and standard
dietary intakes in transitional water			error were not provided.
ecosystems			
Targeted PFAS analyses and	2023	10.1016/j.envint.2022.107640	The necessary data to
extractable organofluorine –			calculate TMF and standard
Enhancing our understanding of the			error were not provided.
presence of unknown PFAS in			
Norwegian wildlife			
Investigation of levels of	2022	10.1007/s11356-021-17236-5	The necessary data to
perfluoroalkyl substances in			calculate TMF and standard
freshwater fishes collected in a			error were not provided.
6			
contaminated area of veneto Region,			

Point source characterization of per-	2019	10.1039/c9em00281b	The necessary data to
And polyfluoroalkyl substances			calculate TMF and standard
(PFASs) and extractable			error were not provided.
organofluorine (EOF) in freshwater			
and aquatic invertebrates			
Diet and metabolic state are the main	NA	10.1016/j.envpol.2017.04.100	Only one species
factors determining concentrations of			investigated
perfluoroalkyl substances in female			
polar bears from Svalbard			
Perfluoroalkyl acids in marine	2009	10.1007/s00244-008-9282-7	The necessary data to
organisms from lake Shihwa, Korea			calculate TMF and standard
			error were not provided.
Fate of perfluoroalkyl substances	NA	10.1016/j.watres.2018.01.066	The necessary data to
within a small stream food web			calculate TMF and standard
affected by sewage effluent			error were not provided.

Other Supplementary Material

Legends for Data S1 - S5:

- Data S1: raw data related to characteristics of the included studies in the meta-analysis
- Data S2: raw data related to parameters of food webs
- Data S3: raw data related to PFAS analytes
- Data S4: quantitative datasets used for effect size calculations
- Data S5: study validity assessment results