ORIGINALE ARTICLE

Influence of Ph on Bioaccumulation of Perfluoroalkyl Substances In *Daphnia Magna* Exposed to Model Proteins

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ABSTRACT

Background: Perfluoroalkyl substances (PFASs) constitute a class of anionic fluorinated surfactants characterized by a perfluoroalkyl chain and carboxylate or sulfonate functional groups. PFASs have seen increased use over the past two decades due to their chemical stability. **Objectives**: Our study aims to assess the influence of different pH levels on the bioaccumulation of six types of PFASs in Daphnia magna, particularly in the presence of bovine albumin and soy peptone. **Methods**: Bioaccumulation experiments were conducted, with proteins prepared at concentrations of 10 mg L-1 in artificial freshwater (AFW). The pH was adjusted to 6.5, 7.5, and 8.5 for each solution. Ten D. magna organisms were introduced into each beaker at the onset of exposure after 72 hours. Dialysis experiments were conducted to investigate the binding of proteins to PFASs, determining the partition coefficients (Kp, L kg-1) of PFASs between protein and water. Finally, PFASs in D. magna were extracted using an ion-pairing agent and analyzed through liquid chromatography–tandem mass spectrometry. **Results**: The findings indicated that pH significantly affects the bioaccumulation of PFASs by Daphnia magna in the presence of proteins under both alkaline and acidic conditions. In the presence of 10 mg L-1 protein, the body burden of PFASs decreased with increasing pH from 6.5 to 8.5. The decreasing ratios of PFASs body burden in Daphnia magna ranged from 27% to 58% for soy peptone and 28% to 72% for bovine albumin. Consequently, it can be concluded that the body burden of PFASs at acidic pH was higher than that at alkaline pH conditions. **Conclusions**: The study suggests that the bioaccumulation of PFASs in Daphnia magna is influenced by growth, body weight, and pH conditions in the system. Additionally, partition coefficients (Kp) of PFASs between protein and water increased with the elongation of PFAS chain length for all treatments in different pH conditions. However, Kp values decreased as pH increased from 6.5 to 8.5, attributed to a reduction in electrostatic interaction.

***KEYWORDS**: Perfluoroalkyl substances (PFASs), Daphnia magna, Bioaccumulation, Protein, Dissolved organic matter (DOM), pH.*

1. INTRODUCTION

Perfluoroalkyl substances (PFASs) have been synthesized for over fifty years and find extensive applications in various industrial and consumer products. They are commonly used as stain, water, and grease repellents in carpets and clothing, as well as nonstick coatings in cooking utensils [1]. Widespread in aquatic systems [2,3,4] and detected in humans and animals [5], PFASs exhibit persistent traits both in the environment and within the human body, often taking years to clear [6]. Similar to many organic pollutants, PFASs pose toxic effects, including liver damage, increased fetal mortality, and are suspected to be carcinogenic [7].

PFASs demonstrate a propensity for bioaccumulation in aquatic organisms and a tendency to bind to proteins within these organisms. For instance, serum albumin in birds and fishes serves as a primary binding pool for PFOS [8]. Studies by Giesy et al., (2002) reported PFOS concentrations of 2.5 mg ml-1 in bald eagle serum and 3.6 mg g^{-1} in mink liver [9]. Another study [10] focused on PFOS bioconcentration in fish in aquatic systems, revealing concentrations up to 1 mg L-1. Consequently, PFASs are sometimes considered proteinophilic compounds [11]. Proteins, ubiquitous in organisms, including animals and plants, are also prevalent in aquatic systems, originating from wastewater in various processing industries, such as abattoir, whey, casein, fish, and vegetables [12].

In our previous work (Xia et al., 2013), we provided evidence that proteins in the aquatic environment can combine with PFASs, reducing their bioavailability [13]. A relatively higher concentration of proteins (> 1 mg L^{-1}) was found to decrease the bioaccumulation of PFASs in D. magna. Furthermore, aquatic environmental conditions significantly impact the conformation of protein molecules, subsequently affecting their environmental functions, such as interactions with organic pollutants. Ionizable residues in amino acids have been shown to play vital roles in protein binding to other molecules and in enzyme mechanisms, exerting considerable influence on protein structure, stability, and solubility [14,15,16]. The changing value of pH can modify the protonation state of a protein residue, coupling with a conformational change of the protein [16,17]. Given these considerations, the pH value can affect the structure and conformation of proteins. Thus, we hypothesize that pH will influence the interaction of proteins and PFASs, subsequently affecting the bioavailability and bioaccumulation of PFASs in aquatic organisms.

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Consequently, the primary objective of this study was to investigate the effects of pH on the bioaccumulation of six types of PFASs in Daphnia magna. These include perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), and perfluorododecanoic acid (PFDoA). Two model proteins, bovine albumin from animals and soy peptone from plants, were compared, and the influence of pH was examined. Additionally, the study explored the binding of PFASs to protein compounds under different pH values and calculated the freely dissolved concentrations of PFASs to unravel the mechanisms by which pH affects the bioaccumulation of PFASs. This study marks the first report on the effect of pH on the bioaccumulation of PFASs by organisms in water in the presence of protein compounds.

2. MATERIALS AND METHODS

2.1 Reagents

PFOA (99.9%) and PFDA (99.9%) were procured from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA); PFUnA (95%), PFNA (97%), and PFDoA (95%) from Acros Organics (NJ, USA); PFOS (98%) from Tokyo Chemical Industries (Tokyo, Japan). A purity-corrected equimass stock standard solution containing the PFASs was prepared in an 80:20 (v/v) methanol/water solution with a concentration of 200 mg L-1 for each PFAS. Chromatography-grade methanol was obtained from J.T. Baker (Phillipsburg, NJ, USA). [1,2,3,4-13C4] perfluorooctanoic acid (MPFOA) (purity > 99%) and [1,2,3,4-13C4] perfluorooctane sulfonate (MPFOS), used as recovery indicators, were obtained from Wellington Laboratories (Guelph, Canada). Ammonium acetate (98%), methyl-tert-butyl ether (MTBE, 99.5%), and tetrabutylammonium hydrogensulfate (TBA) were procured from Sigma-Aldrich Chemical Co. (St. Louis, MI, USA) and used for PFAS extraction from D. magna. Bovine albumin and soy peptone were purchased from Sigma-Aldrich (St. Louis, MI, USA) and Organotechnie (La Courneuve, France), respectively. Anhydrous sodium hydrogen phosphate (Na2HPO4) and sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) were acquired from Fisher Chemical (Fairlawn, NJ, USA). Spectra6 dialysis bags with a molecular weight of 7000 Da were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MI, USA). Hydrochloric acid (HCl, 37%), sodium hydroxide (NaOH, 99.99%), calcium chloride ($CaCl_2$), and sodium chloride (NaCl) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2 *Daphnia magna* cultivation

D. magna were cultured under conditions described in the guideline of Organization for Economic Cooperation and Development for the testing of chemicals [18]. Briefly, the *D. magna* were cultured in artificial freshwater (AFW) containing 0.294g $CaCl_2$, 0.123g $MgSO_4$, 0.064g $NaHCO_3$, and 0.006g KCl per liter of deionized water (with Ca^{2+}=2.64 mmol L^{-1}, Na^{+}=0.76 mmol L^{-1}, and pH=7.15), and they were maintained at 21 ± 0.5°C under a 16: 8 (light: dark) photoperiod. Cultured *daphnids* were fed a suspension of *Scenedesmus subspicatus* twice a day. The detailed culture procedure has been described in Dai et al., (2013) study [19].

2.3 Effect of pH on the bioaccumulation of PFASs in the presence of proteins

The bioaccumulation experiments were carried out in 500 ml polypropylene beakers. Each type of protein was prepared with a concentration of 10 mg L-1 in AFW, respectively. A total of 200 ml protein solution was added to each beaker, followed by the addition of 0.02 mL of PFAS solution (50 mg L-1) with a nominal concentration of 5 µg L-1 for each PFAS. Methanol was employed as a carrier, and its quantity in the final test medium was less than 0.1 ml L-1, having no toxic effect on D. magna. Subsequently, 0.1 mol L-1 NaOH and HCl were utilized to adjust the pH values to 6.5, 7.5, and 8.5 for each solution, respectively. D. magna is known to thrive in water with a pH ranging from 6 to 9 [20]. The beakers were placed on a shaker at 95 rpm in the dark for 72 h. Afterward, a total of 10 D. magna (with an average wet weight of 2.07±0.10 mg) were added to each beaker and cultured at 21 ± 0.5°C under a 16:8 (light: dark) photoperiod for 72 h. Subsequently, the 10 D. magna were transferred from each beaker using a pipette to a polystyrene culture dish and rinsed with AFW. The rinsed D. magna were dried with filter paper and then transferred to a 10 ml centrifuge tube, where a wet weight was determined. The D. magna were then frozen and stored at -20 °C until extraction. A control group was established to examine the bioaccumulation of PFASs in the absence of protein, and a blank experiment without spiking PFASs and protein was also conducted. Each experiment set was performed in triplicate. The pH and hardness of the experimental solutions were measured at the beginning and end of all tests, and the results indicated that their variations were ⩽ 5%. Despite no food being provided to the D. magna during the test period, all the daphnids survived after the bioaccumulation experiments. The bioaccumulation factors (BAF, L kg-1) of PFASs at the end of bioaccumulation experiments were calculated according to equation (1):

$$BAF = \frac{C_B}{C_W} \quad (1)$$

Where:

C_B is the PFAS concentration in *D. magna* (µg kg^{-1}) at the end of the experiment;
C_W is the PFAS concentration in water phase (µg L^{-1}).

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2.4 Dialysis bag experiments

Dialysis bag experiments were conducted to investigate the binding of protein to PFASs. Before the experiment, dialysis bags were cut into 8 cm lengths and placed in boiling deionized water for 5 minutes, after which they were washed with deionized water (at 60~80 °C) followed by additional rinses at room temperature (twice). A total of 20 mL of bovine albumin or soy peptone stock solution with a concentration of 10 mg L^{-1} was added to each dialysis bag. The bags were sealed and placed into 200 ml of deionized water in 500 ml polypropylene beakers. They were then positioned on a shaker at 90 rpm in darkness for 72 hours to remove components with a molecular weight less than 7000 Da.

The pre-treated dialysis bags containing protein solutions were transferred to beakers with 200 ml of AFW. Specific amounts of PFASs solution with nominal concentrations of 5 μg L^{-1} were added to these dialysis bags. Subsequently, the pH of the solution (both inside and outside of the dialysis bag) was adjusted to 6.5, 7.5, and 8.5, respectively, for each type of protein. The dialysis bags were resealed, and the beakers were again placed on a shaker at 120 rpm in darkness at 21°C for 7 days. Each experiment consisted of three replicates.

After reaching equilibrium, water samples were collected from the inside and outside of the dialysis bags for the determination of PFASs. The partition coefficients (Kp, L kg^{-1}) of PFASs between protein and water were calculated using the following equation

$$K_p = \frac{C_s}{C_{free}} \quad (2)$$

Where:

***C_{free}* (μg L^{-1})**: is the freely dissolved concentration of each PFAS in the solution of PFASs and protein, equal to the PFAS concentration outside the dialysis bag;
***C_s* (μg kg^{-1}):** is the PFAS concentration bound with protein, which was calculated based on the difference of PFASs between inside and outside of the dialysis bag.

2.5 Extraction and analysis of PFASs

The PFASs in *D. magna* and water samples of the dialysis bag experiments were extracted by an ion-pairing agent extracted technique with slight modification [21,22]. In brief, 2 mL of Na_2CO_3 (0.25 mol L^{-1}), 1 mL of the ion-pairing agent TBA (0.5 mol L^{-1}, adjusted to pH 10), 2 mL of MTBE, and 100 μL (10 ng) of MPFOA and MPFOS (the internal standards) were added into each polypropylene centrifuge tube containing 10 *D. magna* or 4.5 mL water sample. The detailed procedure has been described in the previous study [19]. PFASs were analyzed using liquid chromatography–tandem mass spectrometry (LC–MS/MS; Dionex Ultimate 3000 and Applied Biosystems API 3200) in electrospray negative ionization mode. A 10 μl aliquot of sample was injected into a 4.6 x150 mm Acclaim 120 C18 Column, and the mobile phase was 50 mmol L^{-1} ammonium acetate and methanol with a flow rate of 1 mL min^{-1}. The detailed procedure can be found in Xia et al., (2012) previous study [19].

2.6 Quality assurance and quality control

The determined limits of quantification (LOQs) (S/N=10) for the target analytes using LC-MS/MS fell within the range of 0.01-0.05 μg L^{-1}. Moreover, all procedural blank areas were consistently found to be less than half of the LOQs. The correlation coefficients of the standard calibration curves consistently exceeded 0.99, and the repeatability of these calibration curves was systematically verified before each set of determinations. Recoveries of the target analytes (MPFOS and MPFOA) from both water samples and protein solutions were within the robust range of 86% to 108%. Similarly, recoveries of the target analytes from *D. magna* organisms ranged from 81% to 95%, while the recoveries of MPFOS and MPFOA—mass spectrometric isotopes spiked into *D. magna* samples—were robustly between 90% and 95% (Table S1). A comprehensive procedure for quality assurance and quality control is elucidated in the Supporting Information, and all data underwent corrections based on the recovery indicators.

The mass balance results of the dialysis bag experiments revealed that the variations in PFASs within the system were consistently less than 10%. Additionally, the results from the control experiment demonstrated that the difference in PFAS concentration between the inside and outside of the dialysis bag was consistently less than 8% in the absence of protein. PFASs were not detected in the *D. magna* samples from the blank bioaccumulation experiments. Furthermore, each PFAS accumulated in *D. magna* constituted less than 2% of the total spiked amount in the system, affirming that the bioaccumulation of PFASs in the organisms did not substantially alter the nominal concentrations of PFASs in the water system or disrupt the equilibrium of PFASs between protein and water.

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Table S1: Analytical parameters and recoveries of PFASs.

Analyte	Molecular structure	Log K_{ow}[a]	Log K_{oc}[b] (SE, n=3)	Primary and production ions(m/z)	Daphnia tissue recovery (%)	Water sample recovery (%)
PFOS	$C_8F_{17}SO_3H$	5.25	2.57(0.13)	498.9>79.8	83± 2	86±9
PFOA	$C_7F_{15}COOH$	4.30	2.06	412.9 > 369.0	95±4	86±9
PFNA	$C_8F_{17}COOH$	4.84	2.39(0.09)	463.0 > 418.9	81±3	93±5
PFDA	$C_9F_{19}COOH$	5.30	2.76(0.11)	512.9 > 468.9	85±5	108±5
PFUnA	$C_{10}F_{21}COOH$	5.76	3.30(0.11)	562.9 > 518.9	88±1	108±2
PFDoA	$C_{11}F_{23}COOH$		-	612.9 > 569.0	82±5	104±2
MPFOA	[1,2,3,4-$^{13}C_4$] perfluorooctanoic acid			416.8 > 371.8	95±2	90±5
MPFOS	[1,2,3,4-$^{13}C_4$] perfluorooctane sulfonate			503.0 > 79.9	90±2	91±6

[a] *n*-octanol/water partition coefficient, data from Arp, H.P.H., Niederer, C., Goss, K.U., 2006. Predicting the partitioning behavior of various highly fluorinated compounds. Environ. Sci. Technol. 40, 7298-7304.

[b] Log K_{oc} data from Higgins, C., Luthy, R., 2006. Sorption of Perfluorinated Surfactants on Sediments. Environ. Sci. Technol. 40, 7251-7256.

2.7 Statistical data analysis

All statistical analyses were performed using SPSS 18.0 for windows (SPSS Inc., Chicago (IL)., USA). Analysis of variance (ANOVA, one factor) was carried out to test differences between each two compared groups. Duncan's multiple range test was used to test the differences among compared groups. Difference was considered significant when the significance level was smaller than 0.05.

3. RESULTS AND DISCUSSION

3.1 Effects of soy peptone and bovine albumin on PFAS bioaccumulation

According to the results shown in Figure 1, the decreasing ratios of body burden of PFASs caused by bovine albumin (28~72%) were higher than those caused by soy peptone (27~58%) in the protein concentration (10 mg L^{-1}) in the systems and the decrease was significant for PFUnA and PFDoA ($P< 0.05$). For most kinds of PFASs, the body burden of 10 mg L^{-1} bovine albumin group was significantly lower than soy peptone including the control group ($P< 0.05$). It can be inferred that bovine albumin with a higher molecular weight had more effect on the bioaccumulation of PFASs by *D.magna* than soy peptone in water (Table S2) with presence of different pH conditions. We observed the similar result in our previous work Xia et al., (2013) [23]. Furthermore, the results showed that the fraction of high molecular weight of protein plays an important role on the bioaccumulation of PFASs when compared to the low molecular weight of protein and both protein inhibited the bioaccumulation.

Table S2 Elemental composition of bovine albumin and soy peptone used in this study

Physico-chemical characteristics	Bovine albumin[a]	Soy peptone[b]
Molecular weight (Da)	66.5kDa	80% <7 kDa and 20% >7 kDa
Total Amino acids	607.0	494
Total nitrogen (TN) (%)	16.0	8.8 -10.1
α- amino nitrogen (AN) (%)	-	3.0 - 3.8
AN/TN x 100	-	30 - 43
Chloride (as NaCl) (%)	0.9	≤ 3.0
Sulfated ash (%)	≤2.0	≤15
pH of 1% Solution	5.2-7	-
pH (5 % solution)	-	6.6 – 7.6
Overall dimensions (Å)	40 × 140	-
Frictional ratio, f/f_0	1.30	-
Estimated α –helix (%)	54	-
Estimated β-form (%)	18	-
Residue on ignition (%)	-	≤ 18
Loss on drying (%)	-	≤ 11
Intrinsic viscosity (η)	0.0413	-
Diffusion constant, $D_{20,W}$ x10^7	5.9	-
Fatty Acid Depleted	5.3	-
Sedimentation constant, $S_{20,W}$ x 10^{13}	4.5 (monomer), 6.7 (dimer)	-

[a] data provided by SIGMA, 3050 Sprunce Street, Saint Louis, Missouri 63103 USA; [b] data provided by Organotechnie® S.A.S. Edition 2010/02 27, avenue Jean Mermoz, 93120 La Courneuve, France.

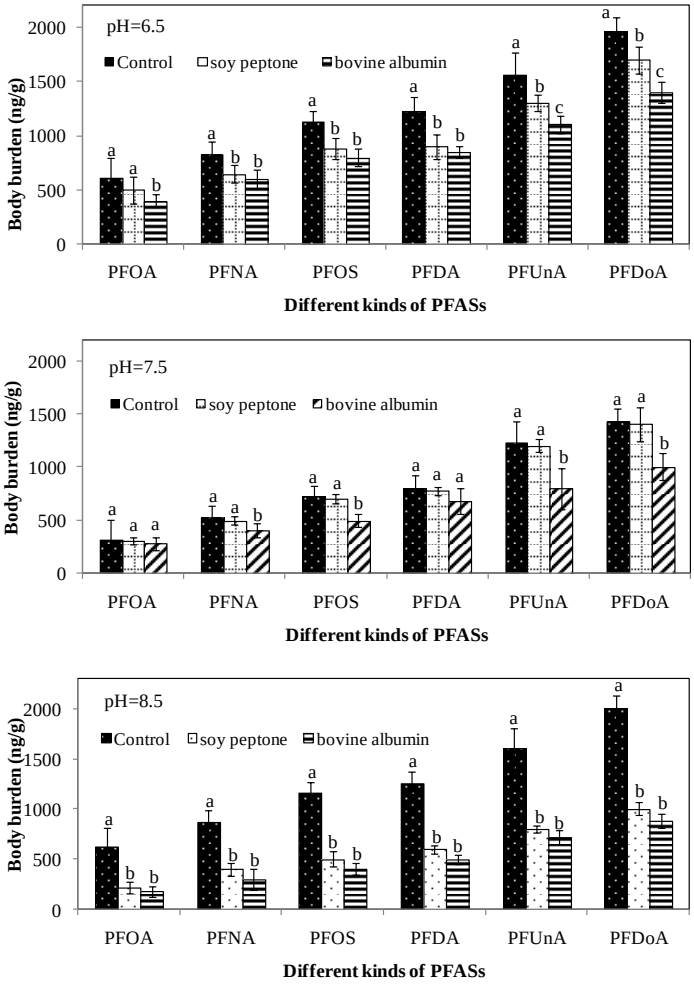

Figure 1: Effects of soy peptone and bovine albumin (10 mg L^{-1}) on the bioaccumulation of PFASs in *Daphnia magna* under different pH conditions (mean±standard deviation, n=3).

3.2 Effect of pH on PFAS bioaccumulation in the presence of proteins

The pH plays a crucial role in adsorption experiments owing to the pronounced reactivity of H^+ (or OH^-) in the solution. The impact of different pH values on the bioaccumulation of PFASs in the presence of proteins is depicted in Figure 1 and Figure 2, encompassing a physiologically relevant range as the medium pH transitions from acidic (pH = 6.5) to basic conditions (pH = 8.5).

As illustrated in Figure 1, the body burden of the protein solution was markedly lower than the control group values (absence of protein) when the pH ranged from 6.5 to 8.5 ($P< 0.05$). Notably, there were no significant differences in the body burdens for PFOA, PFNA, and PFDA ($p>0.05$) at pH=7.5. Since PFASs are ionic organic compounds, prone to ionization under alkaline conditions, the freely dissolved PFASs appear to be facilitated at high pH values. Consequently, bioaccumulation could be inhibited at high pH, as demonstrated by the significantly lower body burden of PFASs at pH 8.5 compared to other pH levels in the control group. For instance, the body burden of PFDoA decreased from 1700±70 to 1000±50 and 1400±27 to 900±25 ng g-1 when the pH increased from 6.5 to 8.5 for soy peptone and bovine albumin, respectively.

Given that PFASs exist in anionic form, electrostatic attraction facilitates the adsorption process. Figure 2 clearly indicates that PFASs adsorption decreases as the pH increases, attributed to the reduction in positive sites on the protein surface. Similar trends have been reported for other minerals [24,25]. In the presence of 10 mg L-1 soy peptone, the body burden and BAFss (Table 1, Table S3) of PFASs decrease with increasing pH, dropping by 2758% when the pH increases from 6.5 to 8.5. For bovine albumin, the trends of body burden and BAFss mirror those in soy peptone solutions, decreasing by 2872%. The body burden of PFASs in pH 8.5 solution is significantly lower than that in neutral or acidic conditions ($P< 0.05$). It appears that the acidic condition benefits the bioavailability of PFASs in protein solutions. Similar trends have been reported by Yu and al., (2009) and Yu et al., (2009) [26,27], who demonstrated that the sorption of PFOS by sediments, activated carbon, and resin decreases with increasing pH. The trends shift in alkaline conditions, inhibiting the bioaccumulation of PFASs for both soy peptone and bovine albumin. Furthermore, the body burden of the bovine albumin group is only about 47% of the control group, significantly lower than other groups ($P< 0.05$).

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In summary, pH exerts a significant influence on the bioaccumulation of PFASs in *D. magna* in the presence of soy peptone and bovine albumin, with both proteins inhibiting bioaccumulation in this scenario. Notably, the body burden in the bovine albumin is significantly lower than in the other groups ($P< 0.05$), as shown in Figure 1. When *Daphnia magna* were exposed to acidic conditions (pH = 6.5), the body burden reached its highest values, while similarly low values were observed under neutral and basic conditions. However, the body burden in the pH 6.5 group is relatively higher. This discrepancy may be attributed to *D. magna's* preference for alkaline conditions [20]. There was a stress response when exposed to acidic conditions, leading to an accelerated respiratory rate, subsequently increasing the uptake of PFASs. An increase in pH resulted in a decrease in the body burden in the adsorption of six kinds of PFASs on protein, likely due to the reduction in electrostatic interaction. The results indicate that the uptake of these compounds diminishes with an increase in pH value, underscoring the significant role of electrostatic interaction in the adsorption of PFASs on protein [28].

Table S3 BAF_{SS} values (L kg^{-1}) of PFASs based on the nominal concentration of PFASs (5μg L^{-1}) under the effect of different pH and types of protein (10 mg L^{-1}).

PFASs	PFOA	PFNA	PFOS	PFDA	PFUnA	PFDoA
Control(pH=6.5)	122±11	166±10	226±14	246±17	312±20	392±26
Control(pH=7.5)	64±3	106±5	145±10	160±11	247±13	286±18
Control(pH=8.5)	126±9	174±7	233±11	250±18	320±15	400±30
pH=6.5 soy peptone	100±14	130±16	176±20	180±23	260±9	340±25
pH= 7.5soy peptone	60±4	100±8	140±21	156±11	240±12	280±23
pH=8.5 soy peptone	44±3	80±6	100±20	120±8	160±17	200±12
pH=6.5 bovine albumin	80±5	120±7	160±6	170±11	220±15	280±19
pH= 7.5 bovine albumin	56±12	80±13	100±11	136±24	160±38	200±25
pH=8.5 bovine albumin	36±2	60±7	80±6	100±8	144±18	176±12

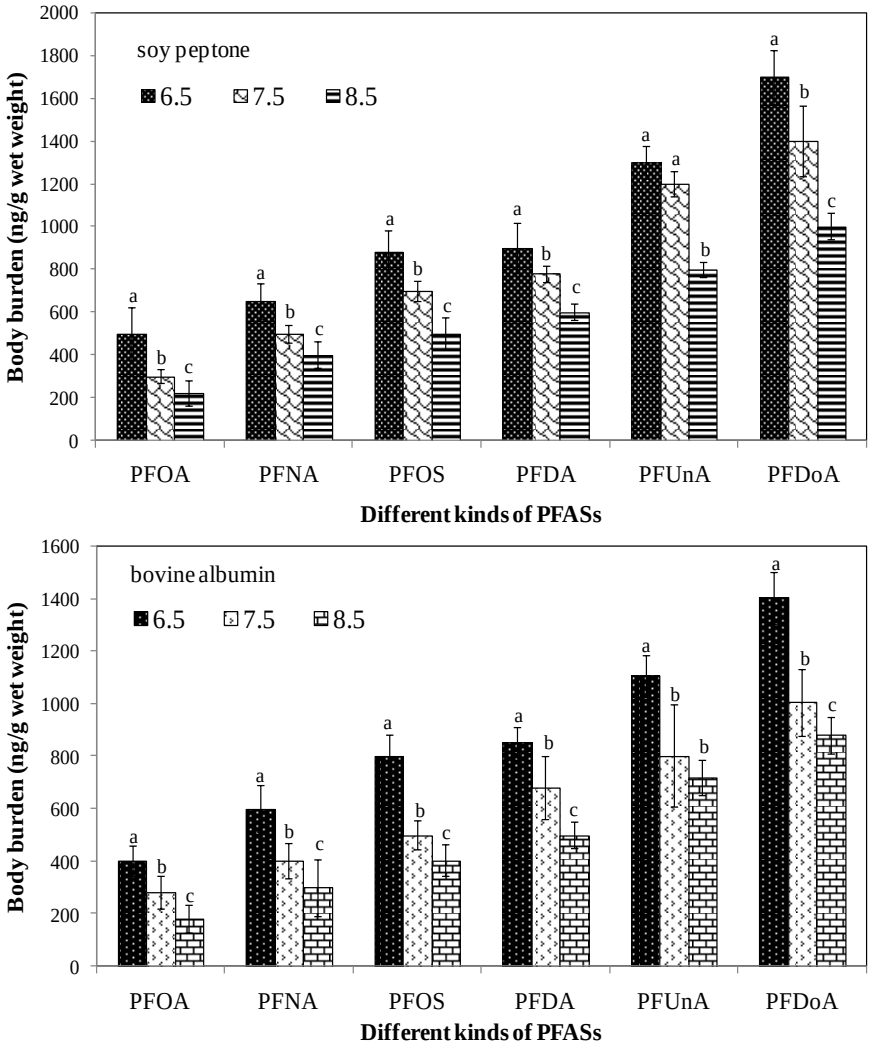

Figure 2: Effect of pH on the bioaccumulation of PFASs by *Daphnia magna* with the presence of soy peptone and bovine albumin (10 mg L^{-1}) (mean±standard deviation, n=3).

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3.3 Influencing mechanism of pH on PFAS bioaccumulation with the presence of protein

3.3.1 Effect mechanism of pH between protein and PFASs on the bioaccumulation in *Daphnia magna*

Based on the results illustrated in Figure 2, an unexpected yet crucial observation emerged from the data analysis: the body burden of PFASs in *D. magna* at acidic pH was significantly higher than at alkaline pH. This implies that the elevated bioaccumulation might interfere with protein intake, potentially impacting the growth and reproduction of D. magna. Furthermore, bioaccumulation was found to be influenced by body weight. When exposed to alkaline conditions, Daphnia magna exhibited an average body weight of 4.46 mg, compared to 2.17 mg under acidic conditions after 3 days of exposure in the system. Under alkaline conditions, *D. magna* increased food uptake, allowing for energy availability and potential PFAS elimination from the organism. This suggests that alterations in *D. magna* in acidified waters could be attributed to the low pH effect on respiration. As a result, air breathers could become numerically dominant, hindering normal growth and leading to increased PFAS uptake. However, despite the increased uptake, *D. magna* faced challenges in eliminating PFASs, evident in the elevated bioaccumulation factor (BAF) values at pH 6.5.

Previous studies, such as Alibone et al., (1981) [29], reported depressed O_2 uptake rates in *D. magna* under low pH conditions, affecting gill function. This decrease in CO_2 diffusion gradient due to increasing acidity may contribute to the observed difficulties in PFAS elimination. Additionally, the ingestion and digestion of protein by *D. magna* may result in the incorporation of protein-bound PFASs into the organism.

Furthermore, the release of perfluoroalkyl substances into the environment poses a risk, with differences in uptake processes between neutral and acidic conditions. The neutral pH condition is more lipophilic than the ionized fraction, leading to easier bioaccumulation at low pH. This pH-dependent bioconcentration factor profile aligns with findings in other organisms, such as *Lemna gibba* and *Scenedesmus sp*. In conclusion, prolonged exposure of *Daphnia magna* to PFASs may disrupt the growth status of these organisms at both individual and population levels, posing risks to aquatic ecosystems.

Shifting the focus to bovine albumin and soy peptone molecules, their structures with various functional groups enable both inter- and intra-molecular repulsion and attraction. The finding that the sorption coefficients (Kp) of bovine albumin and soy peptone at alkaline pH are lower than at acidic pH suggests increased repulsion and protein aggregation at low pH. As pH increases, protein molecules disperse, leading to a decrease in PFAS binding. However, it's important to note that the dispersion of dissolved organic matter can reduce hydrophobic regions, impacting the sorption coefficient of pyrene. For PFAS sorption, various forces come into play due to their hydrophobic and hydrophilic properties. Electrostatic attraction, as highlighted by previous studies, plays a crucial role in PFOS-protein binding, with additional contributions from non-covalent bonds like hydrogen bonds, hydrophobic interactions, and van der Waals forces. Overall, electrostatic interaction depends on PFAS surface charge and protein charge, as reflected in the logKp values being lower in high pH solutions compared to low pH solutions, emphasizing the significant role of electrostatic association in PFAS-protein interactions.

4. CONCLUSIONS

The pH significantly influences the bioaccumulation of PFASs, potentially serving as the predominant factor in determining the extent of adsorption on proteins. Given that the solution composition also affects PFASs' adsorption behavior, special attention should be given to the potential bridging effect initiated by pH. It was observed that PFASs exhibit greater toxicity and bioconcentration at lower pH levels, thereby impacting the bioaccumulation in *D. magna*.

Dialysis analysis revealed a decrease in the binding coefficients of PFASs with bovine albumin and soy peptone as pH increased from 6.5 to 8.5. The results further highlighted that the interaction between PFASs and proteins is not solely governed by hydrophobic forces; electrostatic associations may also play a significant role in binding PFASs.

The obtained results demonstrate that pH has a considerable impact on the PFASs accumulation process during simultaneous exposure to bovine albumin and soy peptone. Additionally, the bioaccumulation of PFASs is controlled by the growth of *D. magna*.

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Supporting Information: Additional information includes the QA/QC procedures, analytical parameters of PFASs, physicochemical parameters of protein and BAFSS values of PFASs.

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