Environmental Science & Technology

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Blood Kinetics of Lipophilic and Proteinophilic Pollutants during Two Types of Long-Term Fast in King Penguins

Margaux Mollier, Paco Bustamante, Ignacio Martinez-Alvarez, Quentin Schull, Pierre Labadie, Hélène Budzinski, Yves Cherel, and Alice Carravieri*

Cite This: Environ. Sci. Technol. 2024, 58, 6138–6148

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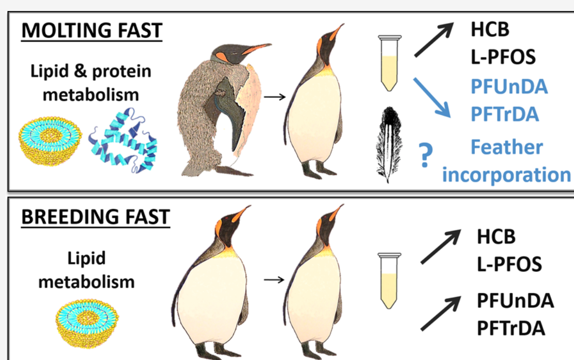
ABSTRACT: In vertebrates, fasting is an intricate physiological process associated with strong metabolic changes, yet its effect on pollutant residue variation is poorly understood. Here, we quantified long-term changes in plasma concentrations of 20 organochlorine and 16 perfluoroalkyl pollutants in king penguins *Aptenodytes patagonicus* during the breeding and molting fasts, which are marked by low and high levels of protein catabolism, respectively, and by strong lipid use. The profile of measured pollutants in plasma was dominated by perfluorooctanesulfonic acid (PFOS, initial relative contribution of 60%). Initial total pollutant concentrations were similar in molting (3.3–5.7 ng g^{-1} ww) and breeding penguins (range of 4.2–7.3 ng g^{-1} wet weight, ww). Long-term fasting (25 days) for molting and breeding led, respectively, to a 1.8- and 2.2-fold increase in total plasma pollutant concentrations, although the rate and direction of change were compound-specific. Hexachlorbenzene (HCB) and PFOS concentrations increased in plasma (net mobilization) during both types of fasting, likely due to lipid use. Plasma perfluoroundecanoate (PFUnDA) and perfluorotridecanoate (PFTrDA) concentrations increased in breeders (net mobilization) but decreased in molting individuals (net excretion), suggesting a significant incorporation of these pollutants into feathers. This study is a key contribution to our understanding of pollutant variation in blood during long-term fasting in wildlife.



KEYWORDS: *Subantarctic, breeding, molt, PFAS, plasma, POPs, seabird, weight loss*

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1. INTRODUCTION

Most persistent organochlorine compounds (OCs) and some perfluoroalkyl substances (PFAS) (i.e., perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS), and related compounds) are regulated by the Stockholm Convention[1] because they are persistent, mobile, biomagnifying, and toxic,[2,3] and are thus key targets of ecotoxicological monitoring worldwide. Marine food webs are particularly threatened by OCs and PFAS,[2,3] which can be found in very high concentrations in marine predators.[4,5] Seabirds have been used effectively to monitor marine contaminants for decades.[4,6,7] Diet is the main exposure route to contaminants in seabirds and feeding ecology is a key extrinsic factor driving variation in their concentrations.[8−10] Variation due to intrinsic traits (e.g., age class, sex) has also received considerable attention,[11−13] while the importance of other physiological variables, such as energy use and metabolic changes, is far less understood.[6,14−16]

Fasting is a common physiological process in the life cycle of seabirds, with most species fasting during breeding, incubation, migration, or molting.[17,18] OCs are lipophilic compounds that are preferentially stored in adipose tissues and mobilized in association with lipid metabolism, as shown in marine mammals and humans.[5,19,20] In seabirds, relationships between body condition (i.e., fat stores) and circulating OC concentrations are often attributed to lipid use.[21,22] However, only three studies, all on the same species, have quantified this directly in fasting individuals, showing a strong increase in plasma OC concentrations in female common eiders *Somateria mollissima* during egg incubation.[23−25] Unlike OCs, PFAS are amphiphilic molecules that have high affinity for proteins, such as plasma albumin, organic anion transporters, and fatty acid binding proteins,[26,27] and tend to accumulate in protein-rich compartments such as plasma, liver, and kidneys.[28−30] PFAS concentrations have been related to body condition in humans,[31,32] wild mammals,[33,34] and seabirds,[35] with mixed results. Yet, to the best of our knowledge, there has been no

Received: December 30, 2023
Revised: March 14, 2024
Accepted: March 14, 2024
Published: March 27, 2024

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https://doi.org/10.1021/acs.est.3c10822
Environ. Sci. Technol. 2024, 58, 6138−6148

prior attempt to measure longitudinal changes in PFAS concentrations during fasting in wildlife.

Molting is another key physiological process in birds, necessary to maintain the quality and function of feathers (flight, waterproofing, thermoregulation, ornament, camouflage).[36] Molting is a well-known driver of changes in blood concentrations of mercury, a nonessential metal that is excreted into feathers while they are bound to the bloodstream during their growth.[37,38] The plumage can also contain OCs[39,40] and PFAS[41−43] as a result of incorporation during feather growth and/or of preen oil and atmospheric deposition onto the feather surface.[39,40,44] Quantifying the changes in OCs and PFAS in blood during molt could thus shed new light on their incorporation into feathers. However, no studies have measured molt-related changes in blood concentrations of OCs or PFAS in seabirds because most species renew their plumage at sea, where they are not accessible.[36] Studying the changes in blood OC and PFAS concentrations throughout fasting and molting is essential to (1) quantify contaminant variation due to intrinsic rather than environmental influences, and thus enhance the use of seabird tissues as bioindicators, and (2) improve our understanding of toxicity risks during fasting.

King penguins *Aptenodytes patagonicus* are exceptional study organisms to evaluate pollutant toxicokinetics during long-term fasting and molt. King penguins' life cycle encompasses two periods of prolonged fasting (approximately 4−5 weeks) on land every year: one for renewing their entire plumage ("molting fast"), and one during courtship ("breeding fast") a few weeks later.[45−48] Both fasting periods are preceded by hyperphagia at sea on similar marine prey (myctophid fish)[49,50] to build up large nutrient stores.[51] King penguins therefore experience a natural alternation of periods of obesity and weight loss (up to 58% of their initial body mass),[46] marked by strong physiological changes. The molting fast is associated with a high metabolic rate due to feather synthesis and the decrease in thermal insulation.[47,48] Unlike the breeding fast, the molting fast involves a large mobilization of proteins as well as lipids because amino acids are required for the synthesis of feather keratin.[46,47,52] The breeding fast is characterized by protein sparing and lipid mobilization only to sustain energy requirements. Given the protracted reproductive cycle of king penguins (∼1 year) and individual variation in breeding onset, molting and courtship individuals are present simultaneously in breeding colonies. This offers an ideal opportunity to study blood toxicokinetics of OCs and PFAS during two types of fasting involving the mobilization of different macromolecules under the same temporal and environmental conditions.

The aim of this study was to quantify and compare the change of blood OC and PFAS concentrations throughout 25 days of fasting in molting and breeding wild king penguins from the Crozet Islands, southern Indian Ocean. We tested whether changes were due to pollutant mobilization, pollutant excretion, or body mass loss. We expected: (1) similar initial pollutant concentrations in molting and breeding individuals because of a similar diet in the two groups; (2) a net mobilization of OCs to the blood (plasma) during both types of fast because lipids are the main energy source; (3) a higher mobilization of PFAS to the blood (plasma) during the molting than the breeding fast due to protein breakdown for feather synthesis.

2. MATERIAL AND METHODS

2.1. Study Site and Blood Sampling. This study was conducted from November to December 2014 on molting and breeding king penguins from the Baie du Marin colony, Possession Island (46°25′S, 51°45′E), Crozet Archipelago. Molt takes place in the austral spring after a period of 2−3 weeks of hyperphagia at sea. After molting, birds return to the sea for another period of hyperphagia (2−3 weeks), before returning to land for courtship and breeding. The egg-laying period extends from November to February on the Crozet Islands.[53] King penguins initiating their molt can be easily identified. The first group of molting individuals (N = 12, four males, eight females) was selected based on the wear and tear of the plumage and their visibly high body mass. A second group of breeding male penguins (N = 12) was selected based on their courtship song and their renewed plumage.[54,55] Penguins were captured at the periphery of the colony upon their arrival from the ocean. Each group was housed in open wooden pens of 3 × 4 m within 10 m of the colony. Consequently, the birds were exposed to natural climatic conditions and the ambient sounds of the colony. Birds were individually marked using spray animal dye (Porcimark) and a flipper band (semirigid P.V.C Darvic bands; 25.8 mm wide, 1.9 mm thick, 7.4 g). Birds of both groups were kept captive for 25 days, during which they were regularly weighted and blood sampled (5 mL at days (D) 0, 3, 6, 10, 15, 20, and 25). Body mass change followed closely the known pattern of fasting in king penguins (Section S1 and Figure S1 in the Supporting Information, SI). Blood samples were centrifuged to separate blood cells and plasma within 2 h of sampling and thereafter kept at −20 °C until laboratory analyses. At the end of the experiment, birds were released where captured.

2.2. OC and PFAS Quantification. OCs and PFAS were measured in plasma at the laboratory Environnements et Paléoenvironnements Océaniques et Continentaux, Physico- et Toxico-Chimie de l'environnement (EPOC-LPTC), Bordeaux, France, given their preferential association with plasma lipids and proteins, respectively.[27,56] Targeted OCs included seven indicator polychlorinated biphenyls and 13 organochlorine pesticides and were quantified using gas chromatography coupled with electron capture detection (GC-ECD).[57] Targeted PFAS included seven perfluoroalkyl carboxylic acids (PFCAs), four perfluoralkanesulfonamides, and five sulfonates (PFSAs). PFAS analysis was carried out by online solid phase extraction coupled with high-performance liquid chromatography negative electrospray ionization tandem mass spectrometry.[58] Further details about targeted pollutants, sample preparation, analysis, and quality assurance and quality control are available in the SI (Section S2, Tables S2−S5).

2.3. Data Analysis. Data treatment, figure preparation, and statistical analyses were carried out using R version 4.0.4.[59] Significance was set at α = 0.05 for all tests. Pollutants were included in statistical analyses if at least 70% of the individuals of a group ("molting" or "breeding") had concentrations above the limit of quantification (LQ) throughout the fast (Table S4). For these pollutants, any value below the limit of detection (LD) was replaced by a randomly selected value (*runif* function, *R* environment) in the range between zero and the LD. Similarly, any value below the LQ was replaced by a randomly selected value between the LD and LQ. Substitutions concerned 4,4′-DDE, FOSA, PFNA, and PFTrDA, while HCB, L-PFOS, and PFUnDA were quantified

https://doi.org/10.1021/acs.est.3c10822
Environ. Sci. Technol. 2024, 58, 6138−6148

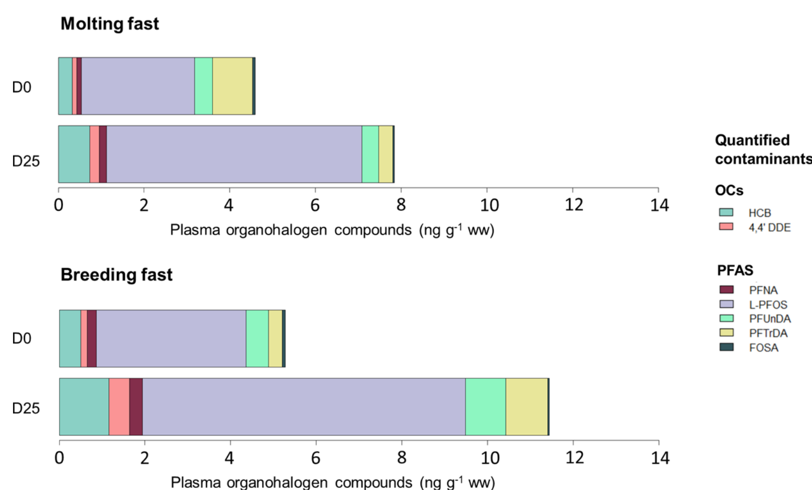

Figure 1. Stacked bar plot of organohalogen compounds (OCs and PFAS) in plasma at D0 and D25 of the molting (N = 9) and breeding fasts (N = 10) in king penguins from the Crozet Islands. Values correspond to the median concentrations.

in 100% of individuals throughout both types of fast (Table S4). Differences in pollutant concentrations between molting and breeding individuals were tested at D0 and D25 through Mann−Whitney tests. The latter approach was also used to test differences in plasma pollutant concentrations between males and females at D0 and D25 in the molting group (Table S6). Given weak sexual differences, the small sample size, and the lack of females in the breeding group, the effect of sex was not included in the following steps of the statistical analysis. In seabirds, sexual differences in contamination are often the consequence of sexual differences in diet or feeding areas.[10,60] Sexual differences in contamination can also result from contaminant excretion into the egg(s), but that effect is usually weak in long-lived species that lay a single egg over ≥12 months.[12]

Pollutant concentration changes throughout fasting were tested in two steps.

First, we applied a mixed model analysis of variance on paired data (*rstatix* package), with individual identity as a random factor, to check for significant differences in plasma concentration between days for each type of fast. The normality of model residuals was checked through QQ plots and Shapiro−Wilk tests, while the homogeneity of variances was checked with plots of model residuals versus fitted values and Levene tests. Posthoc Tukey multiple comparison tests (Tukey honestly significant difference, HSD) were used to know which days were significantly different from each other.

Second, we tested whether observed changes in pollutant concentrations throughout each type of fast were due to net mobilization and accumulation in blood, net excretion from blood, or mass loss alone. To this end, we modeled the *predicted concentration* of a compound that each individual would have if the quantity of the compound circulating in blood was constant throughout the fast-related decline of body mass. The predicted concentration on day t (C_t) was calculated with the following equation: $C_t = \frac{C_0 \times M_0}{M_t}$, where C_0 is the plasma pollutant concentration at D0, M_0 is the body mass of the individual at D0, and M_t is the body mass of the individual on day t. This calculation assumes that the ratio between blood mass and body mass remains constant while fasting, as shown by unchanged hematocrit throughout fasting phase II in king penguins.[45,46] Predicted and observed concentrations were compared each day through paired-sample t tests, for each type of fast, after checking for normality (QQ plot and Shapiro−Wilk test) and homoscedasticity (Levene test). We interpreted observed changes in plasma concentrations throughout fasting as a result of (i) mass loss alone, if there was no difference between predicted and observed concentrations; (ii) net mobilization from internal tissues into the blood and subsequent accumulation there, if the observed concentrations were higher than the predicted ones; and (iii) net excretion from blood (toward other tissues or excrements), if the observed concentrations were lower than the predicted ones. Similar predicted and observed concentrations (interpreted as mass loss dependency) could also arise from equal amounts of pollutants being mobilized into and excreted from the bloodstream.

3. RESULTS

3.1. OC and PFAS Concentrations in Plasma of King Penguins. Among the 36 targeted OCs and PFAS, 26 were detected in king penguins' plasma (Figures S2−S4). Among OCs, only hexachlorbenzene (HCB) and 4,4' dichlorodiphenyldichloroethylene (DDE) concentrations were included in statistical analyses because they had a quantification frequency above 70% in both fasting groups. Among PFAS, linear PFOS (L-PFOS), perfluorooctane sulfonamide (FOSA), perfluorononanoate (PFNA), perfluoroundecanoate (PFUnDA), and perfluorotridecanoate (PFTrDA) had a quantification frequency above 70% in both fasting groups and were the only PFAS included in statistical analyses. Branched PFOS (Br-PFOS), perfluorodecasulfonate (PFDS), perfluorooctanesulfonamidoacetic acids (FOSAA, MeFOSAA, and EtFOSAA), and PFOA were not detected. Perfluoroheptasulfonate (PFHpS) and perfluorohexanesulfonic acid (PFHxS) had high quantification frequencies (≥80%) only on D25 in both fasting groups. Perfluorodecanoate (PFDA) had a high quantification frequency during the breeding (90%) but not the molting fast (≤50%). Perfluorododecanoate (PFDoDA) and perfluorotetradecanoate (PFTeDA) quantification frequency decreased during the molting fast and increased during the breeding fast (up to 80 and 50%, respectively, Figure S2, Table S3).

The profile of measured pollutants in plasma was dominated by PFAS (relative contributions: molting individuals: 90.6 and 87.8%, at D0 and D25, respectively; breeding individuals, 87.3

https://doi.org/10.1021/acs.est.3c10822
Environ. Sci. Technol. 2024, 58, 6138−6148

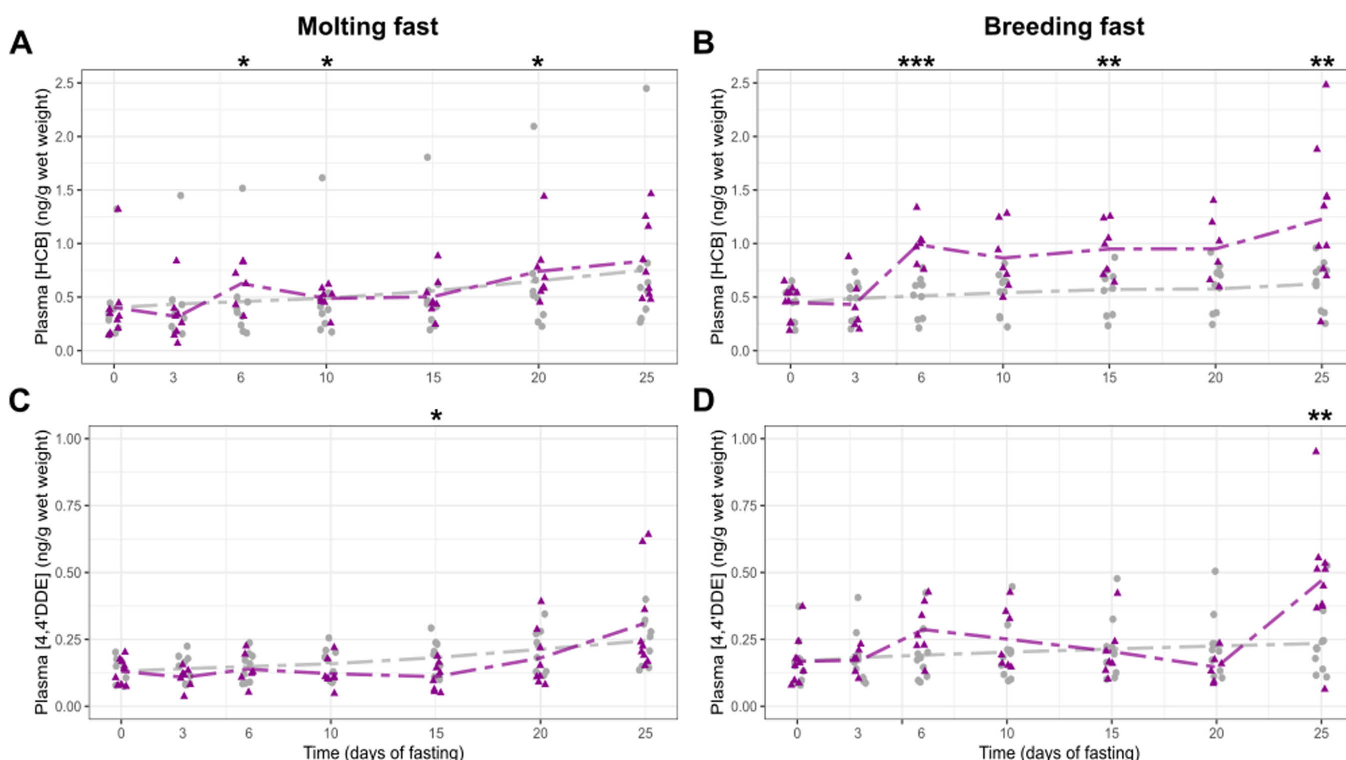

Figure 2. Predicted (gray) and observed (purple) concentrations of HCB (A, B) and 4,4′-DDE (C, D) in plasma of king penguins form the Crozet Islands throughout the molting (left) and breeding fasts (right). Significant differences between the daily mean of predicted and observed values are represented by stars (t tests, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

and 85.6%, at D0 and D25, respectively; Table S4). L-PFOS was the dominant pollutant throughout both types of fast (median concentrations; molting: 2.64 and 5.96 ng g^{-1} ww, at D0 and D25, respectively; breeding: 3.5 and 7.5 ng g^{-1} ww at D0 and D25, respectively; Figure 1, Tables S2 and S7). HCB contributed more than 4,4′-DDE to the total pollutant burden in both groups (median concentrations; molting: 0.32 and 0.73 ng g^{-1} ww at D0 and D25, respectively; breeding, 0.50 and 1.16 ng g^{-1} ww at D0 and D25, respectively; Figure 1, Tables S2 and S7). At D0, PFUnDA and PFTrDA had large relative contributions to the pollutant burden in both groups (up to 9.8 and 20.5%, respectively, Figure 1, Table S7), yet they decreased by a factor of 2−5 in molting birds at D25 (Table S7).

At D0, molting and breeding individuals had similar total pollutant concentrations (Wilcoxon test, $W = 2048$, $p = 0.48$, Figure 1, Table S2). In contrast, total pollutant concentrations were 1.5 times higher in breeding than in molting individuals at D25 ($W = 1640$, $p = 0.01$, Figure 1, Table S2). No significant differences were observed between the two groups at D0 and D25 for HCB and 4,4′-DDE concentrations (W ranged 25−33, all $p > 0.05$). Conversely, PFTrDA concentrations at D0 were higher in molting than in breeding individuals ($W = 80$, $p = 0.003$), while the opposite was true for L-PFOS concentrations ($W = 11$, $p = 0.004$). At D25, L-PFOS concentrations were similar in breeding and molting individuals ($W = 29$, $p = 0.21$), while PFUnDA and PFTrDA concentrations were significantly higher in breeding penguins (both $W = 1$, $p < 0.001$). Within the molting group, males and females had similar organohalogen compound concentrations at both D0 and D25, with two exceptions: males had lower plasma PFTrDA concentrations at D0 and higher plasma 4,4′-DDE concentrations at D25 than females (Table S6).

3.2. Plasma OC Concentrations during the Breeding and Molting Fasts—Observed Data. Observed HCB concentrations increased significantly throughout both the molting ($F_{ANOVA} = 3.75$, $p = 0.0039$) and breeding fasts ($F_{ANOVA} = 5.61$, $p < 0.001$) (Figure S3A,B). HCB concentrations were significantly higher at D25 than D0 and D3, for both groups (Tukey HSD; all $p < 0.05$). HCB concentrations were also significantly higher at D6 than D0 ($p = 0.049$) in breeding individuals. 4,4′-DDE concentrations changed significantly throughout the molting ($F_{ANOVA} = 4.78$, $p < 0.001$) and breeding fasts ($F_{ANOVA} = 6.73$, $p \leq 0.0001$), with concentrations higher at D25 than all other days (all $p < 0.05$) except D20 for the molting, and D6 for the breeding fasts (Figure S3C,D).

3.3. Plasma OC Concentrations during the Molting and Breeding Fasts—Predicted Data. During molt, observed HCB concentrations were significantly higher than predicted ones at D6, D10, and D20 (t ranged from 2.53 to 2.95, all $p < 0.05$), while they were similar the other days (Figure 2A). During the breeding fast, observed HCB concentrations were higher than predicted ones from D6 to D25, with significant differences at D6 ($t = 7.18$, $p < 0.001$), D15 ($t = 3.80$, $p < 0.01$), and D25 ($t = 3.51$, $p < 0.01$) (Figure 2B). During molt, observed and predicted 4,4′-DDE concentrations were similar throughout the fast, except at D15 when observed concentrations were significantly lower than predicted ones ($t = -3.03$, $p < 0.05$, Figure 2C). Conversely, in breeders, observed 4,4′-DDE concentrations were significantly higher than predicted ones on D25 ($t = 4.15$, $p < 0.01$, Figure 2D).

3.4. Plasma PFAS Concentrations during the Molting and Breeding Fasts—Observed Data. Observed L-PFOS concentrations increased throughout both the molting (F_{ANOVA}

https://doi.org/10.1021/acs.est.3c10822
Environ. Sci. Technol. 2024, 58, 6138−6148

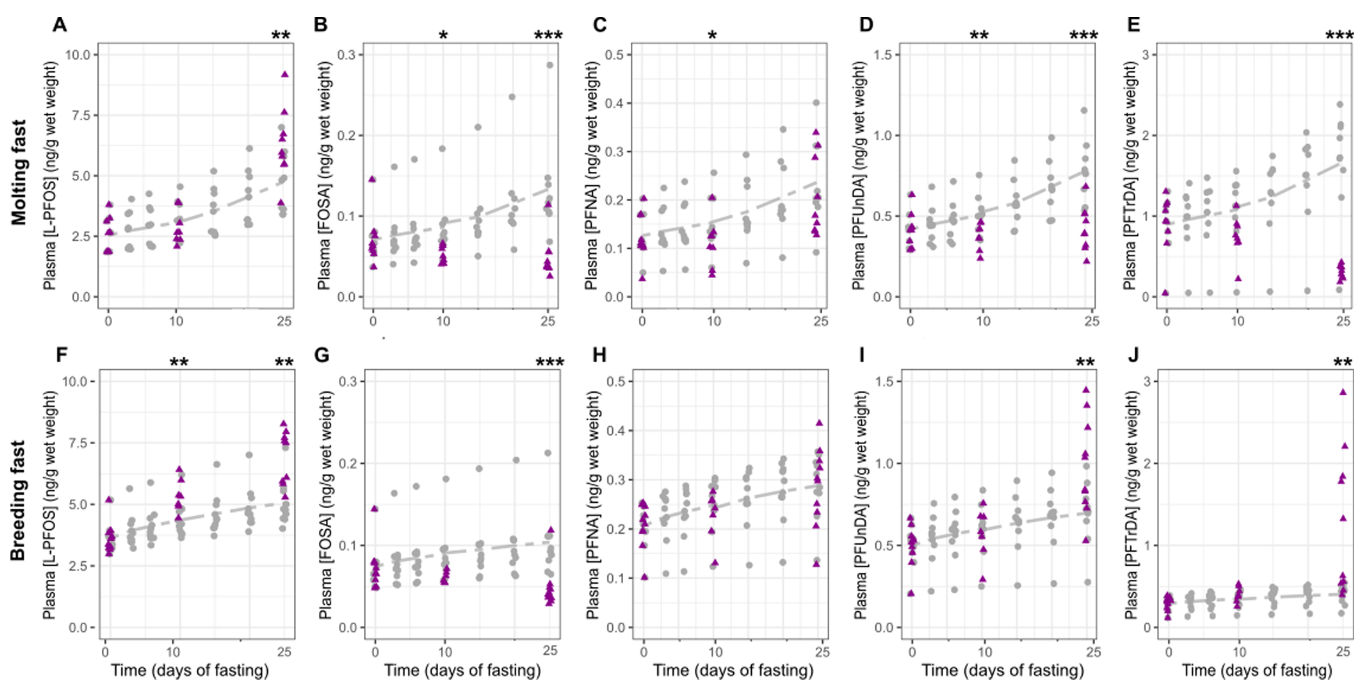

Figure 3. Predicted (gray) and observed (purple) concentration of L-PFOS, FOSA, and three PFCAs (PFNA, PFUnDA, and PFTrDA) in plasma of king penguins from the Crozet Islands during the molting (A−E) and breeding fasts (F−J). Significant differences between the daily mean of predicted and observed values are represented by stars (t tests, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

= 36.48, $p < 0.001$; D0 = D10 < D25, $p < 0.01$) and the breeding fasts ($F_{ANOVA} = 30.08$, $p < 0.001$; D0 < D10 < D25, Tukey HSD, $p < 0.01$) (Figure S4A,F). FOSA concentrations tended to decrease during both types of fast but not significantly so (breeding, $F_{ANOVA} = 3.09$, $p = 0.064$; molting, $F_{ANOVA} = 1.87$, $p = 0.177$, Figure S4B,G). PFNA concentrations increased at the end of the molting fast ($F_{ANOVA} = 5.60$, $p = 0.012$; D0 = D10 < D25, $p < 0.05$ Figure S4C) and throughout the breeding fast ($F_{ANOVA} = 4.25$, $p < 0.001$, D0 ≤ D10 ≤ D25, $p < 0.001$, Figure S4H). No significant changes were observed for plasma PFUnDA concentrations for molting individuals ($F_{ANOVA} = 0.501$, $p = 0.612$, Figure S4D), while they increased significantly at D25 of the breeding fast ($F_{ANOVA} = 16.2$, $p < 0.001$, D0 = D10 < D25, all $p < 0.001$, Figure S4I). PFTrDA concentrations changed significantly during both types of fast, but in opposite directions; molting individuals had lower concentrations at the end of the fast ($F_{ANOVA} = 11.49$, $p < 0.001$; D0 = D10 > D25, all $p < 0.01$; Figure S4E), while breeding individuals had higher concentrations ($F_{ANOVA} = 11.11$, $p < 0.001$; D0 = D10 < D25, all $p < 0.01$; Figure S4J).

3.5. Plasma PFAS Concentrations during the Molting and Breeding Fasts—Predicted Data. Observed L-PFOS concentrations were higher than predicted ones at D25 of the molting fast ($t = 4.25$, $p < 0.01$; Figure 3A) and at D10 and D25 of the breeding fast ($t = 5.34$ and 4.14, both $p < 0.01$; Figure 3F). Conversely, observed FOSA concentrations were significantly lower than predicted ones at D25 during both types of fast ($t = -8.78$ and -6.16, both $p < 0.001$; Figure 3BG). Observed PFNA concentrations were slightly but significantly lower than predicted ones at D10 of the molting fast ($t = -2.45$, $p < 0.05$, Figure 3C), while they were similar to predicted ones throughout the breeding fast (t ranged between −1.88−1.66, $p > 0.05$, Figure 3H). Observed PFUnDA concentrations were significantly lower than predicted ones throughout the molting fast (D10, $t = -4.35$, $p < 0.01$; D25, $t = -6.64$, $p < 0.001$) (Figure 3D), while they were higher than predicted ones at D25 of the breeding fast ($t = 3.98$, $p < 0.01$, Figure 3I). Similar results were observed for PFTrDA, with significantly lower observed than predicted concentrations at the end of the molting fast ($t = -6.07$, $p < 0.001$, Figure 3E) and significantly higher observed than predicted concentrations at the end of the breeding fast ($t = 3.12$, $p < 0.01$, Figure 3J).

4. DISCUSSION

This is the first study to quantify longitudinal changes in blood PFAS concentrations during fasting in wildlife, and the first report of plasma PFAS contamination in king penguins (Munoz et al.[58] erroneously refer to king penguins, while samples were collected on Adélie penguins *Pygoscelis adeliae*; YC personal communication). Long-term fasting for molting and breeding led to a moderate increase in the total plasma OC and PFAS concentrations. Yet, concentrations changed in different directions and rates depending on the compound and type of fast. The two fasting groups differed mainly in changes of plasma PFAS concentrations, suggesting a significant transfer of long-chain PFCAs from blood to feathers during molt. This needs direct confirmation from feather PFCA quantification but is consistent with previous studies documenting large proportions of long-chain PFCAs in feathers.[41−43,61]

4.1. Plasma Concentrations and Profile of OCs and PFAS in King Penguins. The initial total OC and PFAS concentrations were overall comparable in breeding and molting individuals, and between sexes in the molting group, likely following similar dietary exposure[49,50] (confirmed by comparable stable isotopic values in the two groups and sexes, data not shown). Blood OC concentrations can reflect exposure over a few days to several weeks, depending on the compound,[62−64] while PFAS have long blood half-lives[65] (230 days in chicken for L-PFOS).[66] Initial PFUnDA and PFTrDA

https://doi.org/10.1021/acs.est.3c10822
Environ. Sci. Technol. 2024, 58, 6138−6148

concentrations were different between groups, possibly because of blood bioaccumulation along the year in breeding individuals and excretion into feathers in molting individuals (see Section 4.3). A (potentially weak) sex effect cannot be excluded since molting males and females had different plasma PFTrDA concentrations. When compared to other seabirds, king penguins had relatively low OC and relatively high PFAS concentrations, given their ecology and feeding grounds (see Section S3 for detailed comparisons with the literature). Interestingly, PFAS contributed more than OCs to the total pollutant burden of king penguins' plasma, contrary to results in other Southern Hemisphere seabirds, where PFAS contamination is still low, particularly at high latitudes.[67−70] The PFAS profile was consistent with most other studies in seabird plasma, showing a strong contribution of L-PFOS, and of odd-numbered long-chain PFCAs.[8,35,71] King penguins remain within the limits of the Southern Ocean year-round, where they feed almost exclusively on myctophid fish.[72] Our results thus suggest that myctophid fish might be largely contaminated by PFAS in the Southern Ocean, as recently shown in the northeast Atlantic Ocean.[73] This urges direct investigation, given the pivotal role of myctophid fish in Southern Ocean food webs.[49]

4.2. Changes in Plasma OC Concentrations during the Molting and Breeding Fasts. Changes in plasma OC concentrations in king penguins were overall consistent with the hypothesis that prolonged periods of mass loss result in the mobilization of OCs from fat tissues to plasma where they accumulate, as previously shown in seabirds,[16,23,24] marine mammals,[5,19,74] and humans.[20] Both HCB and 4,4′-DDE concentrations increased more during the breeding (factor of 2.7 and 2.9 between D0 and D25, respectively) than the molting fast (factor of 2.1 and 2.4, respectively). The weaker increase in plasma OC concentrations during the molting fast could also be linked to partial excretion into feathers.[39] Net mobilization was clear for HCB throughout the breeding fast. In contrast, prolonged lipid metabolism seemed to be necessary for a net mobilization of the more hydrophobe 4,4′-DDE (log K_{ow}, octanol−water partition coefficient, of 6.5 vs 5.8 for HCB),[75] which was clear only at D25 of the breeding fast. Increasing observed 4,4′-DDE concentrations early during the breeding fast and throughout the molting fast were likely mass-dependent, i.e., (i) they were the result of the concentration of the same quantity of 4,4′-DDE in a smaller volume of blood or (ii) equal amounts of 4,4′-DDE were mobilized into and excreted from blood. The quicker mobilization of HCB than 4,4′-DDE could stem from a larger HCB burden in fat tissues and/or to differences in physicochemical properties between the two OCs. HCB is a smaller, less lipophilic molecule than 4,4′-DDE,[62] which could be easily released into the circulation from the early stages of lipid metabolism. Conversely, more lipophilic compounds are less efficiently mobilized from fat tissues and are strongly concentrated within them during fasting, as shown in marine mammals.[19,74] Contrary to our findings, previous results from incubating common eiders showed a stronger mobilization of 4,4′-DDE than HCB into the circulation.[23−25] Moreover, the rate of change of plasma 4,4′-DDE residues was larger in common eiders than in breeding king penguins (8.2- vs 2.9-fold increase, respectively), despite similar fasting duration (∼20 days) and similar initial plasma 4,4′-DDE concentrations. The strong increase in plasma 4,4′-DDE residues was attributed to large 4,4′-DDE burdens in common eiders' fat tissues.[23,24] Hence, the different fast-related increase of 4,4′-DDE between common eiders and king penguins points to a small 4,4′-DDE burden in king penguins' fat tissues. Conversely, plasma HCB residues showed a stronger increase in king penguins than common eiders (2.7- vs 1.7-fold, respectively; this study and refs 23,24), suggesting that a larger HCB burden was present in king penguins (HCB was previously quantified in Antarctica penguins' fat).[76] Here, king penguins were released toward the end of fasting phase II, when fat stores were still available.[45,46] There could be a further release of 4,4′-DDE and other OCs with high log K_{ow} (e.g., other DDT metabolites, highly chlorinated PCBs) into the bloodstream at a later stage of fasting.

4.3. Changes in Plasma PFAS Concentrations during the Molting and Breeding Fasts. Changes in PFAS concentrations were partially consistent with the hypothesis of stronger release during the molting than during the breeding fast, although clear differences were observed between compounds. Similarly to OCs, plasma L-PFOS concentrations increased during both types of fasting, leading to significant net mobilization and accumulation into the bloodstream. However, contrary to OCs, the rate of increase of L-PFOS was stronger during the molting (2.5-fold increase between D0 and D25) than during the breeding fast (2-fold increase). This suggests that L-PFOS was released into the circulation in association with both lipid and protein metabolism and that potential incorporation into feathers was weak (but see below). The marked increase of plasma L-PFOS concentrations could have been exacerbated by the biotransformation of FOSA into PFOS,[33,77] which is supported by the significant decrease in observed plasma FOSA concentrations in both fasting groups. Kinetics of the other targeted precursors of PFOS, namely, MeFOSAA, EtFOSAA, and FOSAA, could also have helped in interpretation, but their concentrations were all below detection. Alternatively, FOSA and L-PFOS concentration changes were not related, and the decrease in FOSA residues was derived from excretion mechanisms in other tissues, including blood cells. The distribution behavior of FOSA among tissues is known to be unique among PFAS (e.g., higher affinity for blood cells than for plasma in humans),[78] which complicates the interpretation of results. Another challenging pattern to disentangle was the one of PFNA. Previous studies in seabirds indicate a lipid-dependent behavior of PFNA.[29,35] Here, plasma PFNA concentrations showed a mass-dependent change in both fasting groups, which suggests that PFNA mobilization into and excretion from blood were weak and/or balanced in king penguins.

A central finding of this study was that plasma concentrations of PFCAs with a chain longer than nine carbons, in particular, PFUnDA (C_{11}) and PFTrDA (C_{13}), changed in opposite directions depending on the type of fast: they showed a net excretion during the molting fast and a net mobilization during the breeding fast. The rate of change was particularly strong for PFTrDA, with a 3-fold decrease in observed concentrations during molt and a 4-fold increase during the breeding fast. PFDoDA (C_{12}) and PFTeDA (C_{14}) showed similar patterns for their quantification frequency. PFDA (C_{10}) showed a similar trend that could not be quantified precisely due to the low quantification frequency during molt. Hence, we hypothesize, and discuss hereafter, that long-chain C_{10}−C_{14} PFCAs (i) were mobilized mainly in association with lipid metabolism and (ii) were incorporated into feathers during molt.

https://doi.org/10.1021/acs.est.3c10822
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The increase in plasma $C_{10}-C_{14}$ PFCA concentrations along the breeding fast could be associated with lipid mobilization from adipose tissues, similar to L-PFOS. This agrees with previous results showing high plasma PFSA and C_8-C_{10} PFCA concentrations in wild mammals in poor body condition (cross-sectional studies: fasting vs feeding female polar bears *Ursus maritinus*;[34] lean vs fat Arctic foxes *Vulpes lagopus*[33]). The net excretion observed for $C_{10}-C_{14}$ PFCA in molting but not breeding individuals could stem from several nonexclusive factors. Long-chain PFCAs have strong bioaccumulative potential, because their structure favors biliary enterohepatic recirculation, and are more hydrophobic than short-chain PFCAs, which can be more easily eliminated through urine.[79,80] Urine production could be exacerbated in molting individuals, which lose two times more water than breeding individuals during fasting.[47] However, renal tubular reabsorption of PFAS into the blood has been shown in humans and laboratory mammals and is a key driver of the long blood half-life of PFAS.[79,81] Hence, urinary excretion is unlikely to be the main driver of the difference in $C_{10}-C_{14}$ PFCA concentration changes in molting and breeding individuals. In addition to urinary excretion, other potential elimination routes for PFCAs (and other PFAS) include transfer to growing feathers,[41,43,82] preen oil (mainly PFOS[82]), and the egg(s),[30,83] while biotransformation is thought to be negligible.[3,80] Here, egg transfer can be excluded because all breeding individuals were males, and molt takes place before the onset of breeding in this species.[47,48] The chemical composition of preen oil can vary with breeding status, among other factors,[84,85] and could thus be different between molting and breeding individuals. This could drive differences in PFAS transfer to preen oil in the two fasting groups. However, with the exception of PFOS, PFAS transfer to preen oil is thought to be weak.[82,86] While the total amount of synthesized preen oil could also differ between the two fasting groups, this would likely be negligible when compared to the difference in the total amount of synthesized feathers (approximately 400 g in molting vs 0 g in breeding king penguins).[47] Therefore, feather incorporation appears to be the most likely route explaining the excretion of $C_{10}-C_{14}$ PFCAs from blood in molting but not breeding king penguins. This is in agreement with avian studies showing that long-chain PFCA concentrations are correlated between plasma and feathers, unlike other shorter-chain PFAS.[41−43,61] Here, incorporation into feathers was substantial enough to affect plasma residues of $C_{10}-C_{14}$ PFCAs of molting individuals, possibly because of the large feather mass synthesized at once.[47,87] Interestingly, PFTrDA concentrations in breeding individuals at D0 were significantly lower than those of molting individuals at D0, but similar to those of molting individuals at D25 (similar, nonsignificant trend observed for PFUnDA). Breeding individuals had molted ∼1 month before sampling.[47,48] Conversely, molting individuals had been accumulating pollutants since the previous molt (∼1 year before). These results suggest that PFTrDA, and possibly PFUnDA, accumulated in blood along the annual cycle before being excreted into feathers during molt. Repeated PFAS quantification in the same individuals during two successive reproductive cycles should confirm this.

Unlike PFCAs, the L-PFOS pattern of change during the molting fast did not indicate excretion into feathers, despite PFOS concentrations being usually high in feathers and correlated to those in blood.[41] Feather excretion might be significant but not sufficient to decrease plasma L-PFOS concentrations, likely due to larger burdens and stronger lipid-driven mobilization of L-PFOS compared to PFCAs. Previous studies have shown that long-chain PFCAs are preferentially transferred to the eggs in seabirds,[83] and from maternal blood to the placenta in humans,[88] unlike other PFAS including L-PFOS. This has been hypothesized to stem from selective binding of long-chain PFCAs to low-density lipoproteins involved in egg- and placenta transfers.[28,30,88] We thus hypothesize that $C_{10}-C_{14}$ PFCAs can also bind to proteins involved in feather synthesis and/or directly to keratins, to a larger extent than other PFAS including L-PFOS.

To sum up and conclude, repeated measures of OCs and PFAS in king penguins indicated (i) net mobilization and accumulation in plasma of HCB and L-PFOS in both fasting groups and of 4,4′-DDE, PFUnDA, and PFTrDA in breeding individuals only; (ii) mass-dependent increase in plasma PFNA concentrations in both fasting groups; and (iii) net excretion from plasma of FOSA in both fasting groups and of PFUnDA and PFTrDA in molting individuals only. FOSA concentration changes could also arise from the biotransformation into PFOS. OC toxicokinetics were consistent with previous studies, while we showed for the first time a strong excretion potential of long-chain PFCAs into feathers. To confirm this, it is warranted to quantify these compounds in king penguins' feathers, preen oil, and excrements. A larger sample size could better elucidate the longitudinal change of OC and PFAS concentrations during fasting, notably in relation to body mass loss variation between individuals, as well as potential sexual differences. Profiling of plasma proteins and lipids could also help us disentangle whether plasma pollutant variation is linked to fasting-related changes in levels of specific macromolecules (e.g., low-density lipoproteins, albumin, phospholipids, fatty acids, triglycerides). Increases in some compounds throughout fasting were related to mass loss alone, which calls for caution in the interpretation of monitoring data in seabirds of unknown physiological status. The mobilization and accumulation in plasma of L-PFOS and long-chain PFCAs during fasting is worrying since these highly toxic compounds can be preferentially transferred to sensitive tissues such as eggs and the brain.[29,83] This calls urgently for further studies on the deleterious effects of these compounds on physiology and fitness during periods of prolonged fasting in seabirds, particularly when not associated with molting.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c10822.

> Details, figures, and tables about king penguins' fasting phases and their body mass change; pollutant concentrations, quantification frequency, quality assurance and quality control; limits of detection and quantification; sexual differences in contamination in the molting group; and observed concentrations throughout both types of fast (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Alice Carravieri − *Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-La Rochelle Université, 17000 La Rochelle, France; Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372 CNRS-La Rochelle Université, 79360*

https://doi.org/10.1021/acs.est.3c10822
Environ. Sci. Technol. 2024, 58, 6138−6148

Villiers-en-Bois, France; orcid.org/0000-0002-7740-843X; Email: alice.carravieri@gmail.com

Authors

Margaux Mollier − *Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-La Rochelle Université, 17000 La Rochelle, France*

Paco Bustamante − *Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-La Rochelle Université, 17000 La Rochelle, France;* orcid.org/0000-0003-3877-9390

Ignacio Martinez-Alvarez − *CNRS, UMR 5805 EPOC (LPTC Research Group), Université de Bordeaux, F-33405 Cedex Talence, France*

Quentin Schull − *MARBEC, Université de Montpellier, IFREMER, IRD, CNRS, 34203 Sète, France*

Pierre Labadie − *CNRS, UMR 5805 EPOC (LPTC Research Group), Université de Bordeaux, F-33405 Cedex Talence, France;* orcid.org/0000-0001-7184-6327

Hélène Budzinski − *CNRS, UMR 5805 EPOC (LPTC Research Group), Université de Bordeaux, F-33405 Cedex Talence, France*

Yves Cherel − *Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372 CNRS-La Rochelle Université, 79360 Villiers-en-Bois, France*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.3c10822

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Fieldwork was approved by the Conseil des Programmes Scientifiques et Technologies Polaires of the Institut Polaire Français Paul Emile Victor (IPEV), and procedures were approved by the Animal Ethics Committee of IPEV. This study was funded by the Agence Nationale de la Recherche (project ILETOP ANR-16-CE34- 0005, funding A.C.); IPEV (*N*°109, C. Barbraud; *N*°119, J.P. Robin); the Region Nouvelle Aquitaine (France) through the Excellence Chair ECOMM (funding A.C.) and the platform PLATINE (accredited by the CNRS (REGEF) and the University of Bordeaux). We thank Karyn Le Menach, Amélie Frantz, Patrick Pardon, and Laurent Peluhet for analytical support, and Yves Handrich and Agnès Lewden for field assistance. We also thank Christelle Lopes for helpful suggestions on early steps of the data analysis.PB is an honorary member of the Institut Universitaire de France. We also thank five anonymous reviewers for constructive suggestions on a previous version of this article.

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