

# 1 **Controlled chamber formation of per- and polyfluoroalkyl substances** 2 **(PFAS) aerosols with *Pseudomonas fluorescens*: size distributions,** 3 **effects, and inhalation deposition potential**

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13 **Abstract.** Per- and polyfluoroalkyl substances (PFAS) are recognised as atmospheric contaminants, yet processes governing  
14 their aerosol formation, size distribution, and interactions with atmospheric particle surfaces remain unknown. We investigated  
15 aerosolisation and size-resolved behaviour of 25 PFAS covering short-, medium-, and long-chain perfluoroalkyl carboxylic  
16 acids (PFCA), perfluoroalkane sulfonates, fluorotelomer sulfonates and emerging alternatives. Experiments were conducted  
17 under controlled chamber conditions using a water–organic solvent system, in the absence/presence of the model bacterium  
18 *Pseudomonas fluorescens* seed to investigate the potential influence of microbial presence on PFAS behaviour. Most PFAS  
19 exhibited unimodal mass–size distributions peaking at 0.3  $\mu\text{m}$ , indicating dominant association with the fine mode. Sulfonated  
20 PFAS showed broadly similar aerosol-phase concentrations regardless of carbon-chain length, whereas PFCA displayed  
21 increasing aerosolisation with chain length. Perfluorooctane sulfonic acid (PFOS) showed additional ultrafine enrichment, 6:2  
22 fluorotelomer sulfonate (6:2 FTS) and sodium 4,8-dioxa-3H-perfluorononanoate (NaDONA) exhibited broader size profiles,  
23 suggesting compound-specific effects linked to volatility and interfacial behaviour. *Pseudomonas fluorescens* seed did not  
24 enhance PFAS aerosol concentrations through condensation or heterogeneous uptake onto bacterial particles or shift in modal  
25 diameters, and no enrichment was observed at bacterial size mode, indicating limited PFAS–bioaerosol association under the  
26 tested conditions. Multiple-Path Particle Dosimetry (MPPD) modelling based on the measured size distributions predicted  
27 substantial deposition of the aerosol-bound PFAS in the pulmonary region, particularly for compounds enriched in ultrafine  
28 particles. Our findings indicate that PFAS aerosol behaviour in mixed-solvent systems is controlled primarily by physical  
29 droplet generation and evaporation, with implications for airborne transport and inhalation exposure from contaminated  
30 aqueous sources.

## 31 **1 Introduction**

32 Per- and polyfluoroalkyl substances (PFAS) are synthetic organofluorine compounds widely used in industrial and consumer  
33 applications due to their high thermal stability and strong surface-active behaviour (Glüge et al., 2020). Their persistence,  
34 mobility, and potential toxicity have raised growing environmental and health concerns, particularly given their ubiquitous  
35 presence in water, soil, and the atmosphere (see reviews Evich et al., 2022; Faust, 2023). Atmospheric transport is now  
36 recognised as an important pathway for the redistribution of PFAS on regional and global scales (Barber et al., 2007; Ellis et  
37 al., 2004; Kourtchev et al., 2024; Kourtchev et al., 2025; Schenker et al., 2008), yet the mechanisms by which these compounds  
38 enter and are stabilised in the atmospheric particle phase remain poorly understood.

39 PFAS are surfactants that readily accumulate at air–water interfaces (Schaefer et al., 2019) and adsorb onto mineral (Alves et  
40 al., 2020; Folorunsho et al., 2025), organic (Wanzenk et al., 2023), and biological surfaces (Dai et al., 2023). Their strong  
41 interfacial activity suggests that pre-existing airborne particles could act as carriers or “seeds” for PFAS, facilitating their  
42 transfer from aqueous systems into the atmosphere. Laboratory studies with sea-spray aerosol have shown that PFAS  
43 enrichment in surface films exceeds that of bulk water by several orders of magnitude (Johansson et al., 2019), supporting the  
44 likelihood of interfacial transfer. In the atmosphere, aerosol particles can grow through the condensation of semi-volatile  
45 compounds when ambient vapour pressures fall below equilibrium values, leading to vapour uptake by existing particles  
46 (Romakkaniemi et al., 2011; Stolzenburg et al., 2018). The rate and extent of condensation depend on compound volatility,  
47 surface tension, and relative humidity, as well as particle composition. For surface-active species such as PFAS, adsorption or  
48 condensation at particle interfaces may not only contribute to particle growth but also promote their association with pre-  
49 existing aerosols, effectively turning these particles into carriers for atmospheric transport. Interactions with organic or  
50 biological material may further modify these processes by altering surface energy, hygroscopicity, and the stability of particle-  
51 bound PFAS. Wastewater treatment plants (WWTPs) are of particular interest in this context, as they are recognised co-sources  
52 of both PFAS (Cookson and Detwiler, 2022) and biological aerosols, also known as bioaerosol (Li et al., 2016; Poopedi et al.,  
53 2025; Xu et al., 2020). During aeration and mechanical agitation, fine droplets containing PFAS and microbial material can  
54 become airborne, providing a direct mechanism for PFAS transfer from water to air. Elevated concentrations of PFAS are  
55 frequently detected in WWTP effluents and sludges (Cookson and Detwiler, 2022), and both PFAS and bioaerosol emissions  
56 from aeration tanks are well documented (Ahrens et al., 2011; Li et al., 2016; Lin et al., 2022; Pandamkulangara Kizhakkethil  
57 et al., 2025; Poopedi et al., 2025; Shoeib et al., 2016; Xu et al., 2020).

58 Despite growing evidence for PFAS volatilisation and enrichment during water-to-air transfer (Ahrens et al., 2011; Lin et al.,  
59 2022; Pandamkulangara Kizhakkethil and Kourtchev, 2025; Pandamkulangara Kizhakkethil et al., 2024; Shoeib et al., 2016),  
60 the potential involvement of biological aerosol particles in facilitating PFAS aerosolisation remains unexplored. Literature  
61 indicates that bioaerosols can participate in heterogeneous chemical processes (e.g., Ervens and Amato, 2020; Estillore et al.,  
62 2016), suggesting that similar behaviour may occur for PFAS. Recent work has shown that PFAS can associate with bacterial  
63 cells, primarily through adsorption to cell surfaces and, to a lesser extent, through limited uptake into cell interiors (Dai et al.,

64 2023). Controlled batch and miscible-displacement studies using *Pseudomonas aeruginosa* (Gram-negative) and *Bacillus*  
65 *subtilis* (Gram-positive) demonstrated substantial retention and increased retardation of perfluorooctane sulfonic acid (PFOS),  
66 one of the most studied PFAS, in bacterial-inoculated porous media. While these observations are derived from aqueous and  
67 porous-media systems and cannot be directly extrapolated to the atmosphere, they provide a useful conceptual analogue: if  
68 bacterial cell surfaces in water promote PFAS association, analogous surface-mediated interactions could, in principle, occur  
69 with airborne biological particles. To date, however, no studies have examined PFAS interactions with bioaerosols in the  
70 atmospheric context, highlighting a clear and unaddressed research gap. In principle, microbial cells or their fragments could  
71 either enhance PFAS partitioning into the particle phase through sorptive or condensation processes or inhibit it via electrostatic  
72 or interfacial competition.

73 Understanding whether and how biological matter influences PFAS aerosol behaviour is therefore essential for assessing  
74 emission pathways from engineered systems including but not limited to WWTPs. WWTPs represent one example of  
75 environments in which PFAS and biological aerosols may co-occur, highlighting the broader relevance of understanding  
76 PFAS–bioaerosol interactions, rather than serving as a system directly replicated in this study. Furthermore, PFAS–bioaerosol  
77 interactions may alter the hygroscopicity and atmospheric lifetime of emitted particles, with implications for transport and  
78 deposition.

79 A further uncertainty concerns the atmospheric transport, deposition, and human exposure potential of PFAS, all of which are  
80 intrinsically linked to particle size. Particle size governs the residence time and dispersal range of aerosols (Finlayson-Pitts  
81 and Pitts, 2000), their efficiency of dry and wet deposition (Farmer et al., 2021) and likelihood of respiratory uptake (Tsuda et  
82 al., 2013). Smaller submicron particles ( $<1\ \mu\text{m}$ ) have low gravitational settling velocities and long atmospheric residence  
83 times, allowing efficient transport over long distances, whereas coarse particles are more readily removed from the atmosphere  
84 near emission sources through sedimentation and impaction (Zhang and Vet, 2006). Particle size also governs deposition  
85 behaviour and associated health outcomes: fine and ultrafine particles can penetrate deep into the pulmonary region of  
86 respiratory systems and reach the alveoli, where they are linked to cardiovascular and respiratory morbidity (Pope III and  
87 Dockery, 2006), whereas coarse particles deposit mainly in the upper airways and have been shown to induce inflammation  
88 and allergic responses (Wu et al., 2018).

89 Despite its importance, size-resolved information on PFAS in aerosols remains scarce and not always in agreement (Dreyer et  
90 al., 2015; Ge et al., 2017; Guo et al., 2018; Harada et al., 2006; Lin et al., 2022). Some of these studies considered a limited  
91 number of PFAS, typically a few legacy compounds such as perfluorooctanoic acid (PFOA) and PFOS or employed samplers  
92 with only a few size fractions (e.g.  $n=5$ ), which makes direct comparison with other studies difficult.

93 Reported aerosol size distributions vary with compound class, particle size range, and sampling context. In a semi-rural setting  
94 in Germany, Dreyer et al. (2015) observed PFOA predominantly associated with ultrafine and submicron particles ( $< 0.14$   
95  $\mu\text{m}$ ), whereas PFOS showed enrichment in the supermicron range (1.4–3.8  $\mu\text{m}$ ). Ge et al. (2017) reported ionic PFCAs mainly  
96 associated with fine particles ( $< 0.5\ \mu\text{m}$ ) in indoor environments, while PFOS was enriched in coarse roadside aerosols (2.5–  
97 10  $\mu\text{m}$ ). In contrast, Guo et al. (2018) observed bimodal size distributions in urban aerosols during haze conditions, with PFOA

98 present in both fine (0.4–2.1  $\mu\text{m}$ ) and coarse (3.3–10  $\mu\text{m}$ ) modes and PFOS largely associated with coarse particles. Lin et al.  
99 (2022) further observed compound-specific aerosol mass size distribution patterns, with perfluorobutanoic acid (PFBA) and  
100 PFOA showing variable fine- and coarse-mode enrichment, perfluorobutanesulfonic acid (PFBS) generally coarse-dominated,  
101 and PFOS consistently peaking in the 1–10  $\mu\text{m}$  range.

102 These studies provide important data on PFAS size distribution in aerosols; however, the available information remains too  
103 limited to establish general patterns or identify the controlling mechanisms. Controlled laboratory investigations, though  
104 constrained by simplified conditions and the absence of real-world variability, are therefore needed to disentangle the effects  
105 of PFAS molecular structure and interfacial behaviour from the physical processes governing aerosol formation and droplet  
106 drying. Such information is critical for improving atmospheric fate models and exposure assessments.

107 The aim of this study was to advance understanding of PFAS aerosol formation and size distribution under controlled  
108 laboratory conditions, with particular focus on the potential role of bioaerosols as carriers. In this study, we explore how PFAS  
109 with varying carbon chain lengths and functional groups undergo aerosolisation, both in the absence and presence of the model  
110 bacterium *Pseudomonas fluorescens*. This bacterium was chosen as it is commonly detected in wastewater and wastewater-  
111 impacted matrices, having been isolated from WWTP influent and effluent (including species-level recovery of *Pseudomonas*  
112 *fluorescens*), and from raw sewage of municipal treatment plants (phage isolation targeting *Pseudomonas fluorescens*)  
113 (Luczkiewicz et al., 2015; Sillankorva et al., 2008). *Pseudomonas fluorescens* is a ubiquitous freshwater bacterium, frequently  
114 reported in rivers, lakes, and surface waters, where it occurs in the water column, sediments, and biofilms, supporting its  
115 relevance as a representative organism for aquatic and wastewater-influenced systems (Baum et al., 2009; Batrich et al., 2019).  
116 Moreover, it presents a low biosafety risk, being classified as a non-pathogenic, Risk Group 1 organism suitable for use in  
117 controlled laboratory experiments. Size-resolved PFAS aerosol concentrations were determined for both systems to assess the  
118 influence of molecular structure and biological material on particle-phase behaviour. To the best of our knowledge, this is the  
119 first study to directly investigate PFAS–bioaerosol interactions and resolve their mass size distribution from polar solvent  
120 systems under controlled laboratory conditions.

121 The resulting mass–size distributions were then applied to the Multiple-Path Particle Dosimetry (MPPD) model to evaluate  
122 how aerosol size affects potential respiratory deposition and human exposure. Such modelling provides a quantitative context  
123 for interpreting the potential health relevance of observed PFAS size distributions.

## 124 **2 Materials and methods**

### 125 **2.1 Experimental facility, setup, and conditions**

126 The experiments were performed in the Chamber for Aerosol Modelling and Bio-aerosol Research (ChAMBRe) facility at the  
127 University of Genoa, Italy. The chamber is a stainless-steel vessel with an internal volume of 2.2  $\text{m}^3$ , designed for studies of  
128 particle generation, ageing, and interaction under controlled environmental conditions (Massabò et al., 2018). All the

129 experiments were conducted at ambient pressure and in dark conditions. Temperature and humidity inside ChAMBRé were  
130 continuously monitored and maintained at  $23 \pm 3$  °C and  $40 \pm 6$  %, respectively.  
131 Before each experiment, ChAMBRé was evacuated using a composite pumping system (rotary and root pumps) to achieve an  
132 internal pressure of approximately  $5 \times 10^{-2}$  mbar. The reestablishment of atmospheric pressure was facilitated by introducing  
133 ambient air into the chamber using a five-stage filtration, purification, and drying intake system, which comprised an absolute  
134 HEPA filter and a zeolite trap (Vernocchi et al., 2023). A Waveband Integrated Bioaerosol Sensor (WIBS-NEO, Droplet  
135 Measurement Technologies®) has been incorporated into the ChAMBRé particle monitoring system to quantify bio-aerosol  
136 concentration. The extensive data produced by the WIBS during the ChAMBRé experiments were analysed using custom  
137 software developed in Igor 8.0 (Wavemetrics, Inc.), designed to extract airborne bacteria/bioaerosol concentration and size  
138 distribution within the chamber as a function of time and fluorescence intensity. In parallel, total particle number and size  
139 distributions were monitored in real time using a Scanning Mobility Particle Sizer (SMPS 3938, TSI Inc.) equipped with a  
140 differential mobility analyser (DMA 3081A) and a condensation particle counter (CPC 3750) in the range from 18 to 500 nm  
141 and an Optical Particle Sizer (OPS, TSI 3330) covering 0.3–10 µm range.

## 142 **2.2 Aerosol generation and introduction**

### 143 **2.2.1 PFAS-only experiments**

144 A mixed standard solution containing 25 PFAS was prepared using the EPA-533PAR native analyte mixture supplied by  
145 Wellington Laboratories (Ontario, Canada). The mixture comprised a broad suite of ionic PFAS, including perfluoroalkyl  
146 carboxylic acids (PFCA; C4–C12), perfluoroalkane sulfonates (PFSA; C4, C5, C7 linear, and both linear and branched isomers  
147 of C6 and C8), and several fluorotelomer sulfonates and emerging replacement compounds. Specifically, the analyte mixture  
148 consisted of: 4:2 fluorotelomer sulfonate (4:2 FTS); 6:2 fluorotelomer sulfonate (6:2 FTS); 8:2 fluorotelomer sulfonate (8:2  
149 FTS); hexafluoropropylene oxide dimer acid (HFPO-DA); perfluoro(2-((6-chlorohexyl)oxy)ethanesulfonic acid) (9Cl-  
150 PF3ONS); perfluoro(2-ethoxyethane)sulfonic acid (PFEESA); perfluoro-3-methoxypropanoic acid (PFMPA); perfluoro-3,6-  
151 dioxahexanoic acid (3,6-OPFHpA); perfluoro-4-methoxybutanoic acid (PFMBA); perfluorobutane sulfonic acid (L-PFBS);  
152 perfluorobutanoic acid (PFBA); perfluorodecanoic acid (PFDA); perfluorododecanoic acid (PFDoA); perfluoroheptane  
153 sulfonic acid (L-PFHpS); perfluoroheptanoic acid (PFHpA); perfluorohexane sulfonic acid (PFHxS); perfluorohexanoic acid  
154 (PFHxA); perfluorooctane sulfonic acid (PFOS); perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA);  
155 perfluoropentane sulfonic acid (L-PFPeS); perfluoropentanoic acid (PFPeA); perfluoroundecanoic acid (PFUdA); sodium  
156 dodecafluoro-3H-4,8-dioxanonanoate (NaDONA); 11-chloroeicosafuoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS).  
157 All compounds were diluted to a final concentration of  $0.5$  ng mL<sup>-1</sup> in a mixture of 40:60 (v/v) methanol and ultrapure water  
158 ( $18.2$  MΩ cm) to ensure adequate solubility and minimise losses to container surfaces.

159 The addition of methanol ( $\geq 99.9\%$  (GC), LiChrosolv®, Supelco) was necessary to prevent analyte loss due to sorption onto  
160 the nebuliser container walls and to improve the solubility of long-chain PFAS in water. A 40:60 (v/v) methanol–ultrapure

161 water mixture was used based on prior evidence that methanol additions of approximately 30% (v/v) substantially reduce  
162 PFAS losses to glass (e.g. Mancini et al., 2023) and polymer surfaces, including glass components of the nebulising system;  
163 the higher methanol fraction was used here to provide a conservative margin accounting for additional losses during  
164 aerosolisation and contact with both glass and stainless steel components of the system. Methanol was selected due to its full  
165 miscibility with water, rapid evaporation during aerosolisation, low background PFAS contamination, and established  
166 compatibility with PFAS analytical workflows (Kourchev et al., 2022), and was used consistently to ensure reproducible  
167 aerosol generation conditions.

168 Aerosols were generated using a three-jet Collison nebuliser operated at 5 L min<sup>-1</sup> and introduced into ChAMBRe through a  
169 stainless-steel inlet connected directly to the chamber. Aerosol generation continued for 30 min, followed by a mixing period  
170 of 10 min before sampling, facilitated by the mixing fan installed at the base of the ChAMBRe. The internal fan was operated  
171 at 5 Hz, a setting shown by Massabò et al. (2018) to achieve complete mixing in the ChAMBRe within approximately 2 min.  
172 Experiments were repeated three times and are referred to as Exp 1-3 (no bacteria) below. Aerosol drying prior to chamber  
173 introduction was intentionally avoided to minimise PFAS losses. PFAS are known to interact with surfaces and can partition  
174 during drying, so passing the aerosol through additional tubing or drying devices (e.g., diffusion dryers/denuders) would  
175 introduce unnecessary interfaces and increase the risks of losses. Introducing the wet aerosol directly into the chamber therefore  
176 ensured that the measured composition reflected primary aerosol generation rather than processing artefacts. A simplified  
177 schematic of the experimental setup is shown in Figure S1 of supplement.

### 178 **2.2.2 PFAS and *Pseudomonas fluorescens* experiments**

179 The *Pseudomonas fluorescens* ATCC 13525 (obtained from the American Type Culture Collection, University Boulevard,  
180 Manassas, Virginia, United States) was grown in 30 mL volume of nutrient broth medium. The culture was incubated at 25°C  
181 with continuous shaking in a shaker incubator (SKI 4 ARGOLAB, Carpi, Modena, Italy) at 200 rpm. The growth curve was  
182 monitored by measuring the absorbance at  $\lambda = 600$  nm using a spectrophotometer (Shimadzu 1900) until it reached the  
183 stationary phase (approximately 1) corresponding to about 10<sup>9</sup> cells mL<sup>-1</sup>. Subsequently, 20 mL of the bacterial suspension  
184 was centrifuged at 5000 rpm for 10 min, and the cell pellet was resuspended in 20 mL of sterile Milli Q (MQ) water.

185 The bacteria in MQ were nebulised using Sparging Liquid Aerosol Generator (SLAG) (CH Technologies, USA) with a 0.75”  
186 diameter porous disc and nominal pore size of 2  $\mu$ m. 3 mL of the bacterial suspension with a syringe pump flowrate of 0.4 mL  
187 min<sup>-1</sup> were dripped onto the SLAG porous stainless-steel disk and nebulised inside ChAMBRe with a flowrate of 3.5 L min<sup>-1</sup>  
188 as performed in previous experiments (Gatta et al., 2025).

189 Bioaerosol was allowed to mix for 5 min, followed by addition of PFAS in the same way as described in 2.2.1. Aerosol  
190 generation continued for 30 min, followed by a mixing period of 10 min before sampling. Experiments were repeated three  
191 times and are referred to as Exp 4-6 (with bacteria) below.

### 192 **2.2.3 Blank experiments**

193 Blank experiments were conducted to assess potential contamination or background levels arising from the experimental setup  
194 and to correct for any systematic bias in the measurements. A 40:60 (v/v) methanol–ultrapure water mixture without PFAS  
195 was nebulised using a Collison nebuliser at 5 L min<sup>-1</sup>. Aerosol generation was maintained for 30 min, followed by a 10 min  
196 mixing period prior to sampling. All other experimental conditions were identical to those described in Section 2.2.1. The  
197 blank experiments were repeated three times.

### 198 **2.3 Aerosol generation and introduction**

199 Size-segregated aerosol samples were collected using a Nano Micro-Orifice Uniform Deposit Impactor (NanoMOUDI-IITM,  
200 Model 125B, MSP Corporation, USA) operated at a flow rate of 10 L min<sup>-1</sup>. The NanoMOUDI provided aerodynamic cut-off  
201 diameters of 10.000, 5.600, 3.200, 1.800, 1.000, 0.560, 0.320, 0.180, 0.100, 0.056, 0.032, 0.018, and 0.010 µm.

202 Total suspended particles (TSP) were collected in parallel with a double cone sampler, directly connected to ChAMBRé, at a  
203 flow rate 10 L min<sup>-1</sup>. The total sampling duration for both NanoMOUDI and TSP was 2 hours.

204 PallFlex 2500 QAO-UP quartz fibre filters were used as substrates in both samplers. Due to the unavailability of PallFlex 2500  
205 QAO-UP filters for all experiments, quartz microfibre filters (RVMSFQ47Q90, Mega Systems s.r.l.) were used in TSP  
206 sampling in one of the replicate experiments involving bacteria. All filters were prebaked at 450 °C for 2.5 hours prior to  
207 sampling. Based on recoveries and variability between TSP replicates (RSD < 10%), which was not higher than that observed  
208 for replicates using the same filter type, the use of a different filter brand is expected to have a negligible impact on the results.  
209 Following sampling, aerosol samples were wrapped into aluminium foil (prebaked at 450 °C for 2 hours) avoiding contact  
210 with any plasticware and external environment and stored approximately for 60 days at –20 °C until extraction.

### 211 **2.4 PFAS extraction and analysis**

212 Samples generated in the chamber were extracted and analysed following the procedure described by Kourtchev et al. (2022).  
213 Briefly, filter edges in contact with the sampler gaskets were removed prior to extraction. Each filter was placed in a precleaned  
214 10 mL glass vial (Chromacol 10-HSV, Thermo Scientific) and spiked with 25 µL of an internal standard (IS) mixture  
215 containing 16 isotopically labelled (<sup>13</sup>C) PFAS at 1 ng mL<sup>-1</sup> and three telomer sulfonates (M2-4:2 FTS, M2-6:2 FTS, and M2-  
216 8:2 FTS) at 4 ng mL<sup>-1</sup>. Full names of the isotopically labelled PFAS are shown in Table S1 of supplement. Samples were  
217 extracted twice with 5 mL of Liquid Chromatography Mass Spectrometry (LC-MS, CHROMASOLV™ ≥99.9%) grade  
218 methanol using ultrasonic agitation in a chilled water bath for a total of 40 min (2 × 20 min). The combined extracts were  
219 filtered through PTFE membrane filters (0.45 µm, Iso-Disc PTFE-13-4) into prewashed 10 mL glass vials (Chromacol 10-  
220 HSV) tightly closed with metal screw caps and PTFE septa (Chromacol 18-MS and 18-ST101).

221 Extracts were then reduced in volume to 1 mL under a gentle stream of nitrogen and stored at 4 °C until analysis. On the day  
222 of analysis, 4 mL of Optima™ LC-MS grade water was added to each sample, which was subsequently vortex-mixed and

223 analysed using online SPE consisting of an EQUAN MAX Plus Thermo Scientific™ Vanquish™ UHPLC system equipped with  
224 a Thermo Scientific™ TriPlus™ RSH autosampler, following the method described by Kourtchev et al. (2022). All samples  
225 were analysed within 24 hours of extraction for each analytical batch.

226 Only PFAS detected above the method's LOD (Kourtchev et al., 2022) or above blank levels were considered in the data  
227 interpretation. All data were corrected for chamber dilution to account for the continuous inflow of clean air required to  
228 maintain stable chamber pressure. Direct quantification of aerosol-phase PFAS wall losses was not performed, as it would  
229 require time-resolved aerosol-phase PFAS measurements that are not technically feasible at the concentrations applied, given  
230 the absence of online measurement techniques for ionic PFAS and the need for extended offline sampling to achieve sufficient  
231 analytical sensitivity, especially in size-resolved samples. No correction was applied for potential chamber wall losses. Because  
232 the chamber and associated lines are made of stainless steel, and long-chain PFAS are known to interact strongly with metal  
233 surfaces, additional losses to the walls are likely and may contribute to the overall uncertainty. Moreover, wall-loss efficiencies  
234 may vary with PFAS chain length and could influence absolute recoveries. All experiments were therefore conducted under  
235 consistent chamber configuration and operating conditions to minimise variability in wall-loss behaviour and to allow relative  
236 comparisons within a common experimental framework.

## 237 **2.5 Multiple-Path Particle Dosimetry (MPPD) modelling**

238 For each region of the human respiratory tract (Head, Tracheobronchial region (TB) and Pulmonary region(P), the deposition  
239 efficiency as a function of particle size, was estimated using the MPPD model (version 2.11, Applied Research Associates,  
240 Inc.) (Anjilvel and Asgharian, 1995), using the default human breathing parameters (further details and results are provided in  
241 the supplement file). The MPPD model is based on a framework of semiempirical equations and computational algorithms  
242 that simulates particle deposition in the respiratory tract using anatomical and physiological data. It accounts for species-  
243 specific airway geometry, breathing patterns, and particle characteristics to estimate regional and total deposition under various  
244 exposure scenarios.

245 The deposition flux,  $DF_{r,i}$  ( $\text{pg h}^{-1}$ ) to each region of the human respiratory tract (Head, TB or Pulmonary),  $r$ , for each  
246 nanoMOUDI size bin,  $i$  (assuming constant exposure), was then calculated as

$$247 \quad DF_{r,i} = DE_{r,i} \times C_i \times V$$

248 where  $DE_{r,i}$  is the deposition efficiency,  $C_i$  ( $\text{pg m}^{-3}$ ) is the measured concentration of the target PFAS and  $V$  ( $\text{m}^3 \text{h}^{-1}$ ) is the  
249 human breathing rate. The deposition fluxes for the coarse ( $> 2.5 \mu\text{m}$ ), fine ( $0.1 - 2.5 \mu\text{m}$ ), and ultrafine ( $< 0.1 \mu\text{m}$ ) size  
250 fractions were then calculated by summing across the relevant MOUDI size bins.

## 251 3 Results and Discussions

### 252 3.1 Total suspended particles (TSP)

253 Figure 1 shows concentration of individual PFAS measured in the TSP fraction for the PFAS-only (Exp 1–3) and PFAS with  
254 *Pseudomonas fluorescens*-seeded (Exp 4–6) experiments.

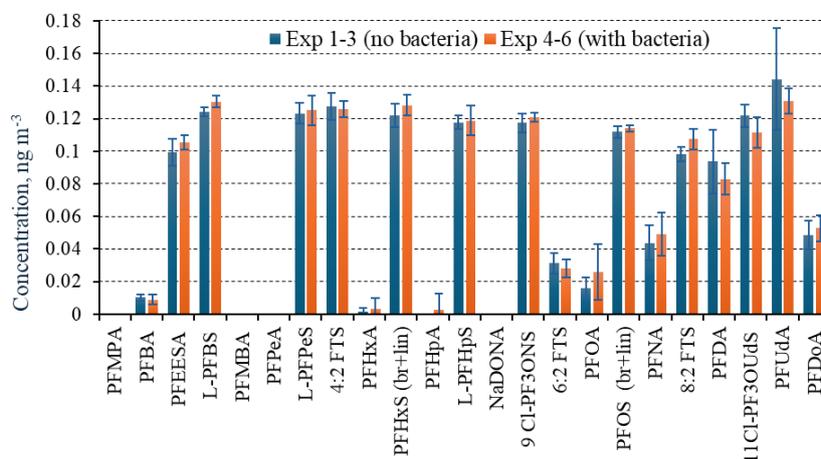
255 Distinct trends were observed between the sulfonated and carboxylated PFAS. The sulfonated compounds, i.e. PFBS, PFHxS,  
256 PFOS, and the fluorotelomer sulfonates (4:2, 6:2, and 8:2 FTS), exhibited relatively uniform aerosol-phase concentrations,  
257 indicating that chain length had little influence on their aerosolisation efficiency. Their consistent behaviour likely reflects the  
258 inherently high surface activity of sulfonates, which promotes their enrichment at the air water interface (Klevan et al., 2025;  
259 Lyu et al., 2022).

260 In contrast, the PFCA showed a clear chain-length dependence, with aerosol-phase concentrations increasing from PFBA to  
261 PFUDA. In a 40:60 methanol-water system, methanol decreases surface tension and solvent polarity, enhancing the solubility  
262 and mobility of longer-chain PFCA relative to pure water (Kutsuna et al., 2012). This mixed-solvent environment therefore  
263 favours the transfer of hydrophobic carboxylates into the aerosol phase. It must be noted that short-chain PFAS e.g. PFBA  
264 (C<sub>3</sub>) are less surface active and can remain in solution (Cai et al., 2022; Klevan et al., 2025). While a clear chain-length-  
265 dependent increase in aerosol-phase PFCA concentrations is observed, any preferential wall losses of longer-chain compounds  
266 would act to reduce their measured recovery and therefore bias the magnitude of this increase towards lower values.

267 Accordingly, sulfonated PFAS appear dominated by interfacial adsorption, whereas carboxylated PFAS are more strongly  
268 affected by bulk-phase solvation governed by solvent composition. While direct comparison with earlier studies is limited by  
269 methodological differences, similar behaviour has been observed during water aeration, where perfluoro sulfonated  
270 compounds exhibited higher aerosolisation efficiencies than carboxylated analogues (Pandamkulangara Kizhakkethil et al.,  
271 2024; Pandamkulangara Kizhakkethil and Kourtchev, 2025). A similar trend, involving an increase in aerosol-phase  
272 perfluorinated alkyl acids, their salts and conjugate bases abundance with perfluoroalkyl chain length but not equivalent  
273 enrichment magnitudes, was also observed under highly aqueous (tap water) conditions in bubble-bursting experiments using  
274 a plunging jet, which is considered representative of nascent sea spray aerosol formation, as reported by Reth et al. (2011).

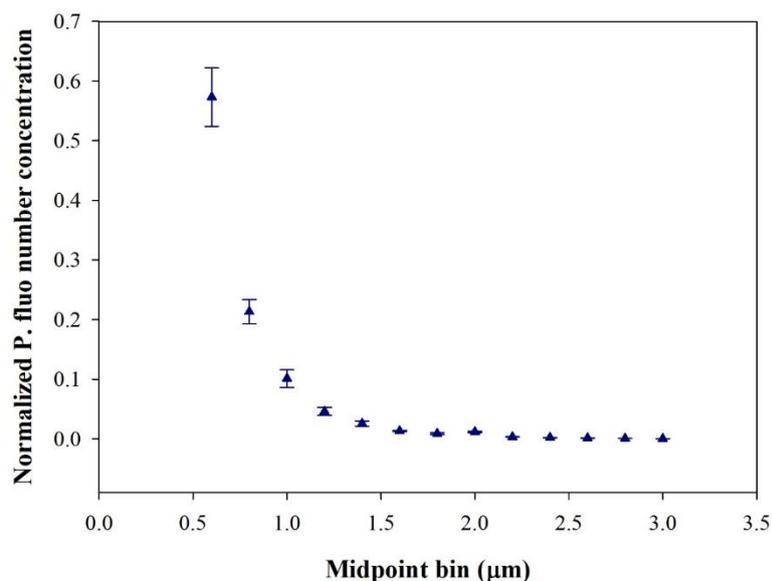
275 In the presence of *Pseudomonas fluorescens* (Fig. 1, Exp 4–6), the overall TSP concentrations of most PFAS were comparable  
276 to those in the PFAS-only experiments, indicating that bacterial seeds did not substantially influence PFAS aerosolisation  
277 under the tested conditions. The average and standard deviation of bacteria concentration across the 3 replicated experiments,  
278 measured by WIBS, 5 minutes after the end of injection was  $29 \pm 1 \text{ # cm}^{-3}$ . Slight reductions observed for some long-chain  
279 PFCA (e.g. PFNA, PFDA, PFUDA) may reflect weak sorptive interactions with bacterial cell walls or their fragments, although  
280 these effects appear minor relative to the dominant physicochemical controls. The overlap of standard deviations between the  
281 two experimental conditions (with and without *Pseudomonas fluorescens*) suggests that PFAS concentrations in aerosol were  
282 similar within the experimental uncertainty.

283 It must be noted that the major fraction of *Pseudomonas fluorescens* present in the chamber was observed around 0.6  $\mu\text{m}$   
 284 (Figure 2), smaller than the typical bacterial dimension (about 2-4  $\mu\text{m}$  in length and 0.5-1.0  $\mu\text{m}$  in diameter). It is worth noting  
 285 that the nebulisation processes exert stress on bacteria, producing fragmentation (Park et al., 2009). Particles in this size range  
 286 lie within the accumulation mode and therefore may act as efficient condensation sinks for condensable species (Engvall et  
 287 al., 2008). In the present experiments, PFAS were introduced in ionic form via nebulisation, yielding PFAS in both the gas  
 288 phase and the particle phase (the latter associated with nebulised droplets and their dried residues). Any enrichment of PFAS  
 289 on the pre-existing bacterial particles would therefore have required gas-particle partitioning to the bacterial surface or  
 290 particle-particle interactions such as coagulation. The absence of measurable enhancement in PFAS aerosol concentrations  
 291 therefore suggests that condensation or adsorption of PFAS onto bacterial surfaces was not thermodynamically favourable, or  
 292 that kinetic limitations prevented significant mass transfer during the experimental timescale. This suggests that, under the  
 293 applied conditions, PFAS aerosol formation and growth were predominantly governed by nebulisation and subsequent droplet  
 294 drying processes rather than by heterogeneous uptake onto biological particles.



295

296 **Figure 1: Aerosol-phase concentrations of individual PFAS measured in total suspended particles (TSP) during PFAS-only**  
 297 **experiments (Exp 1–3, blue) and PFAS + *Pseudomonas fluorescens*-seeded experiments (Exp 4–6, orange). Error bars show the**  
 298 **standard deviation across three chamber replicates with duplicate LC-MS analyses per replicate (n = 6). PFAS are arranged along**  
 299 **the x axis according to carbon chain length. Overall, similar aerosol-phase PFAS concentrations are observed with and without**  
 300 **biological material, indicating no systematic bacterial influence.**



301

302 **Figure 2: Normalised particle size distribution of *Pseudomonas fluorescens* nebulised in ChAMBRé (5 minutes after the end of**  
 303 **bacteria nebulisation). Data represent the mean  $\pm$  standard deviation of three replicate experiments. The dominant fraction of**  
 304 ***Pseudomonas fluorescens* present in the chamber was observed around 0.6  $\mu\text{m}$ .**

### 305 3.2 Mass–Size Distributions of PFAS

306 As mentioned in the Methodology section, not all PFAS detected in the TSP samples were also observed in the NanoMOUDI  
 307 samples, with carboxylated PFAS being mainly affected. This was likely due to their lower aerosol concentrations in the  
 308 chamber (as also observed in TSP) and their generally higher volatility (e.g. PFBA, 6.37 mmHg at 25 °C, Steele et al., 2002;  
 309 PFHxA, 13 mmHg at 25 °C, US EPA, 2012), which reduce particle-phase partitioning. Additionally, “dilution” across multiple  
 310 MOUDI stages may have further contributed to concentrations falling below the LC-MS detection limit in the NanoMOUDI  
 311 samples.

312 The majority of PFAS (excluding 6:2 FTS and PFOS) aerosols exhibited a consistent unimodal mass–size distribution, peaking  
 313 at 0.32  $\mu\text{m}$  (Figure 3). Interestingly, variations in the molecular composition of the tested PFAS, including differences in  
 314 perfluorocarbon chain length and terminal functional group, did not affect the mode diameter of the aerosol mass–size  
 315 distribution. PFAS, representing a broad range of compounds, differ markedly in hydrophobicity and interfacial activity as a  
 316 function of both chain length and functional group (e.g. Lyu et al., 2022; Patel et al., 2024; Leung et al., 2023), which makes  
 317 the present observation somewhat unexpected. For instance, long-chain sulfonates such as PFOS (C8) exhibit considerably  
 318 stronger surface activity than short-chain carboxylates such as PFBA (C3), and their behaviour in bulk aqueous systems differs  
 319 accordingly (Guo et al., 2023).

320 This suggests that, under the applied experimental conditions, aerosol formation and size characteristics were largely governed  
 321 by physical processes. The most plausible explanation is that the aerosol-generation method imposed uniform physical

322 constraints during droplet formation and solvent evaporation, limiting the extent to which molecular properties influenced  
323 particle characteristics. In addition, the use of an organic solvent likely enhanced the solubility of all PFAS, including long-  
324 chain species with low water solubility, allowing them to remain in solution and aerosolise more uniformly during nebulisation.  
325 Methanol substantially reduces surface tension (from 71.7 dyne cm<sup>-1</sup> for pure water to 38.7 dyne cm<sup>-1</sup> at 40 % v/v methanol  
326 at 25 °C; Cheong and Carr, 1987), which promotes droplet formation and minimises differences in surface activity among  
327 PFAS, thereby obscuring potential molecular-specific effects on aerosol behaviour.

328 The two analytes, 6:2 FTS and PFOS, did not follow the general mass-size distribution trend. Although PFOS exhibited a  
329 dominant submicron mass mode at 0.32 µm, similar to other PFAS, its mass-size profile showed a relatively larger fraction of  
330 mass in the smallest measured bins. In other words, PFOS retained the common 0.32-µm residual peak but also displayed  
331 enrichment in the ultrafine fraction compared with other PFAS in the mixture. PFOS may produce a larger fraction of ultrafine  
332 aerosol particles than other PFAS due to its greater surface activity (Klevan et al., 2025; Lyu et al., 2022). Although the  
333 presence of methanol substantially reduces bulk surface tension, PFOS can still dominate dynamic interfacial processes during  
334 rapid droplet formation. Its strong and persistent adsorption at the air–liquid interface, combined with enrichment as methanol  
335 evaporates, likely lowers local surface tension further and inhibits coalescence, resulting in smaller and more stable droplets.  
336 In addition, the anionic nature of PFOS may contribute to electrostatic stabilisation of charged droplets, further enhancing the  
337 ultrafine fraction.

338 Other long-chain PFAS in the mixture did not exhibit similar enrichment, potentially due to competitive adsorption and  
339 intermolecular interactions in mixtures that modulate their effective surface activity.

340 Only a limited number of studies have reported size-resolved mass distributions of PFAS associated with atmospheric particles  
341 from the field observations. Comparison with previous work shows that PFAS size distributions vary considerably among  
342 studies. Harada et al. (2006) and Dreyer et al. (2015) reported compound-dependent patterns, with PFOA and other PFCA  
343 enriched in fine or ultrafine particles, whereas PFOS tended to occur in coarser fractions. In contrast, Guo et al. (2018) found  
344 both PFOA and PFOS primarily associated with fine particles (< 1 µm), while Ge et al. (2017) observed PFCA in ultrafine  
345 particles (< 0.1 µm) and PFOS and other sulfonates in coarse modes. Such variability likely reflects differences in sources,  
346 atmospheric conditions, and sampling methodologies, as well as local physicochemical environments influencing PFAS  
347 partitioning.

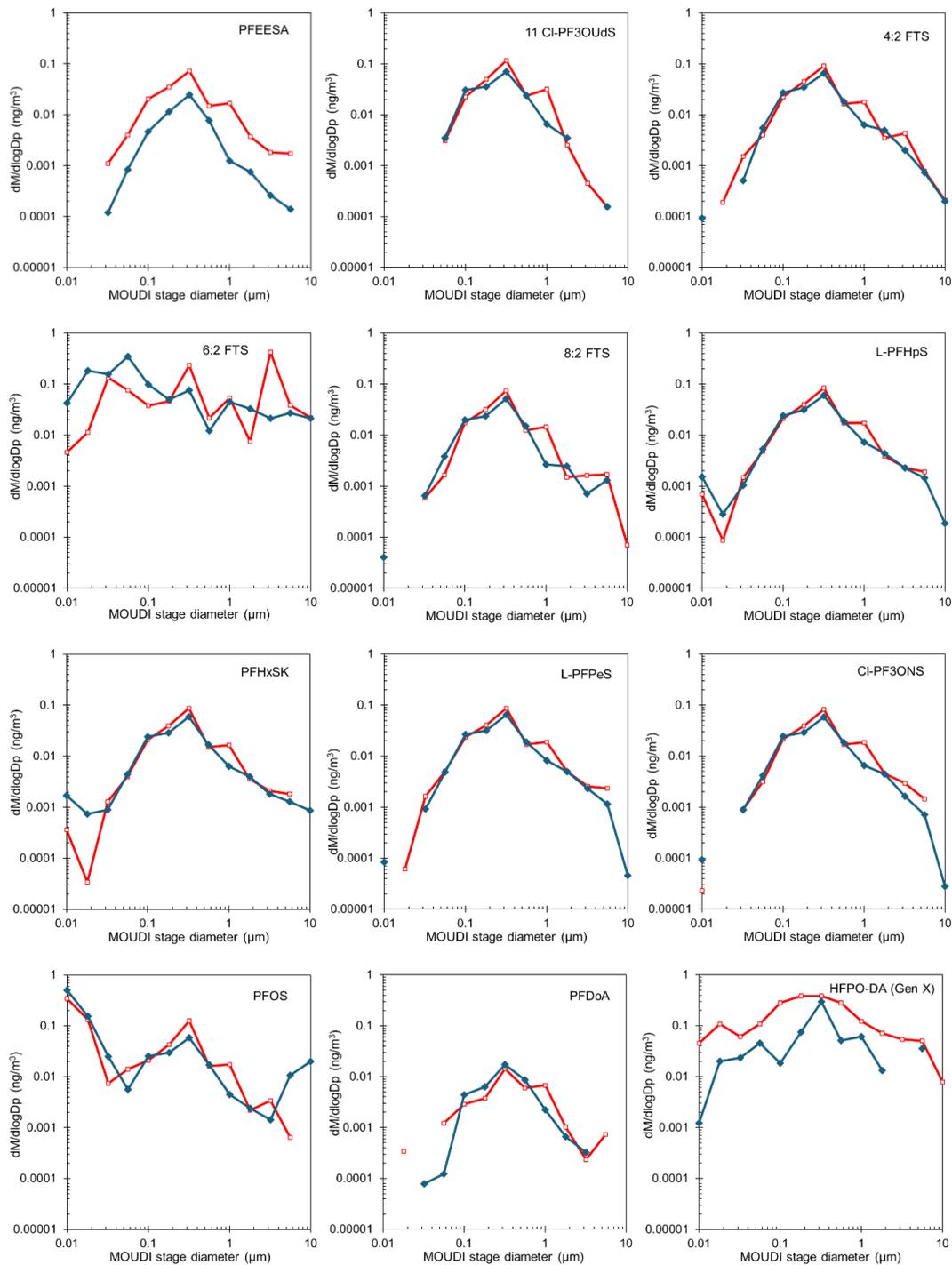
348 To the best of our knowledge, previous laboratory investigations of PFAS aerosol size behaviour have focused primarily on  
349 sea-spray systems, which are not directly comparable to the organic solvent-rich aerosolisation process examined here.  
350 Johansson et al. (2019) reported that the highest enrichment of perfluoroalkyl acids (PFAA) relative to seawater occurred in  
351 aerosols with aerodynamic diameters below 1.6 µm. Sha et al. (2021) found that particle surface-area-to-volume ratio was a  
352 strong predictor of PFAS enrichment in supermicron particles but not in submicron particles, indicating that different physical  
353 controls operate across size ranges. In their subsequent work, Sha et al. (2024) observed that PFAS enrichment was particularly  
354 pronounced in submicrometer sea-spray aerosol particles and varied with chain length and dissolved organic matter content.  
355 These studies suggest that in marine systems, PFAS enrichment and size association are sensitive to experimental conditions

356 and molecular structure, with both particle-scale physics and surfactant properties influencing partitioning. In contrast, the  
357 consistent submicron unimodal distribution observed in the present work likely reflects aerosol formation under organic-  
358 solvent conditions, where rapid solvent evaporation and solute concentration effects impose dominant physical constraints that  
359 reduce the influence of PFAS molecular features on particle size.

360 The introduction of *Pseudomonas fluorescens* into the chamber as a potential seed or carrier of PFAS, in most cases, had either  
361 no effect (within experimental error) or resulted in a uniform decrease in PFAS aerosol mass size concentration across nearly  
362 the entire size range for two analytes (i.e. PFEESA and GenX), while the distribution profile and modal diameter remained  
363 unchanged. As mentioned in the section 3.1, the major fraction of *Pseudomonas fluorescens* in the chamber was observed  
364 around 0.6  $\mu\text{m}$  (Fig 2). The absence of PFAS enrichment at the bacterial modal size ( $\sim 0.6 \mu\text{m}$ ) and the unchanged PFAS modal  
365 diameter ( $\sim 0.3 \mu\text{m}$ ) indicate that *Pseudomonas fluorescens* did not noticeably influence PFAS size distribution under the tested  
366 conditions. This was somewhat unexpected, as PFAS have been shown to associate with bacterial surfaces in aqueous systems  
367 (Dai et al., 2023). The physicochemical environment in aerosols likely may differ from that in bulk water. The outer membrane  
368 of *Pseudomonas fluorescens* carries a net negative charge, arising from acidic functional groups on lipopolysaccharides and  
369 phospholipids (Boyd Chelsea et al., 2014; Charlton et al., 2024), while the tested PFAS are also anionic. Such electrostatic  
370 repulsion could therefore further inhibit PFAS attachment, potentially explaining the absence of observable enrichment at  $\sim 0.6$   
371  $\mu\text{m}$ . In addition, although PFAS are amphiphilic, their molecular structure makes the air–water interface far more favourable  
372 for stabilisation than the hydrated, negatively charged bacterial surface, and PFAS therefore likely preferentially stabilise at  
373 droplet interfaces rather than adsorb onto bacterial cells. Another aspect worth considering is whether *Pseudomonas*  
374 *fluorescens* could have influenced PFAS aerosol concentrations through biochemical transformation rather than solely through  
375 physical carrier processes. In bulk aqueous systems, several *Pseudomonas* species have been reported to partially degrade  
376 sulfonated PFAS, particularly precursors such as H-PFOS, under nutrient-enriched or co-metabolic conditions (e.g., Key et  
377 al., 1998). The latter work involved liquid culture media with high bacterial densities, organic carbon co-substrates, and  
378 prolonged incubation times, which facilitate enzymatic activity and redox transformations. However, even in that study,  
379 evidence for complete degradation of PFOS is lacking; rather, transformation is slow, partial, and often requires co-metabolic  
380 drivers. By contrast, the conditions in our aerosol chamber differ fundamentally. The bacteria were suspended in air with  
381 transient water content (RH $\sim 40\%$ ), rather than immersed in nutrient-rich aqueous media. Under such conditions, the metabolic  
382 activity of *Pseudomonas fluorescens* is expected to be extremely limited. The observed uniform decrease in PFAS aerosol  
383 mass concentration cannot therefore be straightforwardly attributed to microbial degradation, as the air–water interface-  
384 dominated microenvironment is unlikely to sustain enzymatic pathways known to act on PFAS in bulk liquid cultures.  
385 Furthermore, the residence time of particles in the chamber is orders of magnitude shorter than the timescales over which  
386 reported PFAS transformations by *Pseudomonas* occur (days to weeks). It is therefore more plausible that the apparent decrease  
387 in GenX and PFEESA aerosol mass concentrations reflect non-biological processes such as redistribution of material to  
388 chamber or sampler walls, or surface-competition dynamics during condensation, rather than direct microbial influence. If any  
389 biochemical contribution occurred, it would likely be negligible compared with these physicochemical pathways. The

390 difference in TSP concentrations between the *Pseudomonas fluorescens*-seeded (average  $0.105 \pm 0.0043 \text{ ng m}^{-3}$ ,  $n=3$ ) and  
391 unseeded ( $0.099 \pm 0.008 \text{ ng m}^{-3}$ ,  $n=3$ ) experiments for PFEESA was minimal, suggesting that the concentration drop observed  
392 in the NanoMOUDI size-resolved data likely resulted from sampling artefacts or volatility-driven size redistribution rather  
393 than bacterial activity. In this respect, it has been shown that compounds with higher volatility tend to exhibit greater mass  
394 losses through evaporation particularly in impactor-based sampling systems like NanoMOUDI (e.g. Ungeheuer et al., 2022).  
395 Although the same inferences could not be made for GenX from the TSP data, due to high background levels in the TSP  
396 blanks, evaporation from the collection substrate in the NanoMOUDI was likely the dominant loss process, as also suggested  
397 for PFEESA. The relatively high vapour pressure of GenX (2.7 mm Hg at 20 °C, US EPA, 2022) supports this interpretation.  
398 In previous work, it was found that the culturable lifetime of *Pseudomonas fluorescens* in ChAMBRé in dark condition was  
399 about 20 minutes (Gatta et al. 2025). Furthermore, the survival of bacteria in air is known to be sensitive to aerosolisation and  
400 sampling conditions rather than simply liquid-phase growth (Després et al., 2012; Hong et al., 2021). It must be noted that in  
401 our study the bacteria were seeded in sterilised MQ, whereas PFAS were introduced separately via a 40:60 (v/v) methanol-  
402 water nebulisation. Under this experimental setup, bacteria were not subjected to high alcohol strength in the droplet phase;  
403 instead, their methanol exposure was dominated by chamber-average vapour and sporadic interactions near the spray plume.  
404 These conditions are unlikely to produce strong biocidal effects at the population level, so the absence of PFAS enrichment at  
405 the bacterial size mode is better explained by interfacial/partitioning constraints than by methanol-induced loss of viability.  
406 Moreover, even if a fraction of the bacterial population experienced viability loss, this would not preclude potential PFAS  
407 interactions with biological surfaces. Non-viable bacterial cells, cell-wall fragments, and microbial biomass retain abundant  
408 functional groups (carboxyl, phosphate, and amine moieties) known to sorb organic and inorganic species (Fathollahi and  
409 Coupe, 2021; Torres, 2020; Wang and Chen, 2009). Inactivated bacterial biomass is widely used as a biosorbent due to  
410 preserved surface chemistry and polymeric matrices (Torres 2020). In the atmosphere, biological particles occur not only as  
411 intact cells but also as cell fragments and exudates (Després et al., 2012; Fröhlich-Nowoisky et al., 2016), meaning that surface  
412 area and chemical functionality persist even when viability is compromised. Thus, even partial loss of viability would still  
413 permit association of PFAS with microbial surfaces if interfacial partitioning were favourable. The lack of detectable PFAS at  
414 the bacterial size mode therefore reinforces that limited affinity/partitioning, rather than loss of cellular integrity, governed  
415 PFAS–bioaerosol interactions under our experimental conditions. We emphasise that in environmental systems where PFAS  
416 and biological matter may also be internally mixed in aqueous sources prior to droplet formation, different interaction  
417 mechanisms may occur.

418



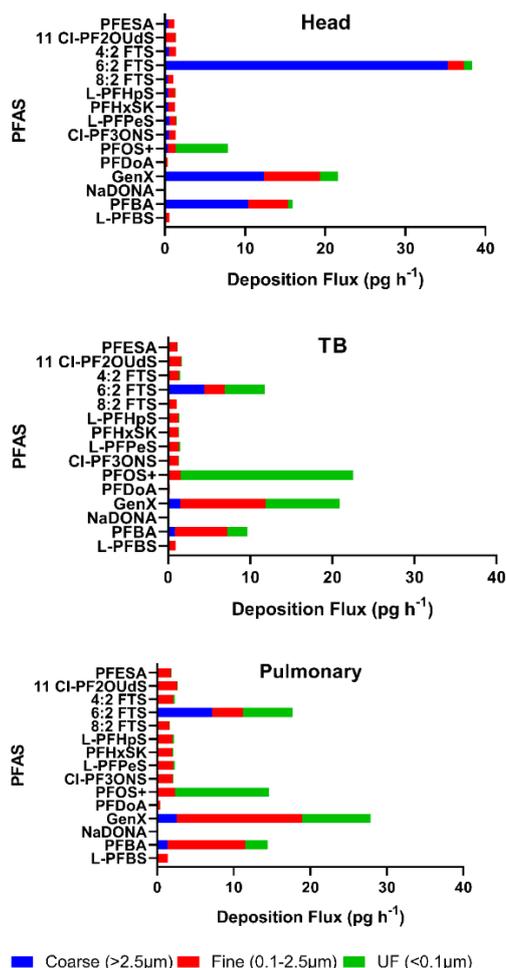
419

420 **Figure 3: Mass size distribution of aerosol from PFAS-only (blue symbols) and PFAS with *Pseudomonas fluorescens* seed (red**  
 421 **symbols) experiments. Most PFAS exhibit a unimodal mass–size distribution centred near 0.3 μm, indicating dominant fine-mode**  
 422 **aerosol association across compounds.**

423

424 **3.3 Multiple-Path Particle Dosimetry (MPPD) modelling**

425 Figure 4 (with data given in the supplement) presents the deposition fluxes across respiratory regions (head, TB, pulmonary)  
 426 and the relative contributions of inhaled PFAS associated with coarse, fine, and ultrafine particles. The majority of PFAS  
 427 measured exhibited similar size distributions, with most particles falling within the fine fraction (0.1 – 2.5  $\mu\text{m}$ ), resulting in  
 428 broadly consistent deposition patterns. The average total deposition flux across these PFAS was  $5.3 \pm 1.0 \text{ pg h}^{-1}$ , with  
 429 deposition distributed relatively evenly between the head ( $23\% \pm 2\%$ ,  $1.22 \text{ pg h}^{-1}$ ), TB ( $26\% \pm 1\%$ ,  $1.34 \text{ pg h}^{-1}$ ), and pulmonary  
 430 ( $40\% \pm 1\%$ ,  $2.08 \text{ pg h}^{-1}$ ) regions.



431

432 **Figure 4: Modelled fractional deposition flux ( $\text{pg h}^{-1}$ ) in the Pulmonary, Tracheobronchial (TB) and Head regions of the human**  
 433 **respiratory tract.**

434 PFDoA and NaDONA were notable exceptions due to their significantly lower aerosol concentrations, resulting in total  
435 deposition fluxes of  $1.18 \text{ pg h}^{-1}$  and  $0.138 \text{ pg h}^{-1}$ , respectively. PFDoA's size distribution was similar to the majority, yielding  
436 comparable regional deposition: 29% to the head ( $0.34 \text{ pg h}^{-1}$ ), 22% to the TB region ( $0.26 \text{ pg h}^{-1}$ ), and 36% to the pulmonary  
437 region ( $0.43 \text{ pg h}^{-1}$ ). NaDONA however exhibited an additional peak in the coarse fraction, shifting deposition toward the head  
438 region, which received 57% of the total flux ( $0.08 \text{ pg h}^{-1}$ ), while the TB and pulmonary regions received 13% ( $0.02 \text{ pg h}^{-1}$ ) and  
439 21% ( $0.03 \text{ pg h}^{-1}$ ), respectively. PFOS and PFBA showed significantly higher aerosol concentrations, with total deposition  
440 fluxes of  $48.1 \text{ pg h}^{-1}$  and  $44.3 \text{ pg h}^{-1}$ , respectively. PFBA had a similar size distribution to the majority but at higher  
441 concentration, resulting in deposition fractions of 36% to the head ( $15.9 \text{ pg h}^{-1}$ ), 22% to the TB region ( $9.7 \text{ pg h}^{-1}$ ), and 33%  
442 to the pulmonary region ( $14.5 \text{ pg h}^{-1}$ ). PFOS, however, had an additional peak in the ultrafine range, shifting deposition toward  
443 the TB region, which received 47% of the total flux ( $22.5 \text{ pg h}^{-1}$ ), while the head region received 16% ( $7.9 \text{ pg h}^{-1}$ ). GenX and  
444 6:2 FTS exhibited the highest aerosol concentrations and deposition fluxes, at  $77.5 \text{ pg/h}$  and  $74.4 \text{ pg h}^{-1}$ , respectively. GenX  
445 followed the typical deposition pattern, with 28% to the head ( $21.6 \text{ pg h}^{-1}$ ), 27% to the TB region ( $20.9 \text{ pg h}^{-1}$ ), and 36% to the  
446 pulmonary region ( $27.9 \text{ pg h}^{-1}$ ). In contrast, 6:2 FTS had additional modes in both the ultrafine and coarse fractions, leading to  
447 a deposition profile skewed toward the head region, which received 52% of the total flux ( $38.4 \text{ pg/h}$ ), while the TB and  
448 pulmonary regions received 16% ( $11.8 \text{ pg h}^{-1}$ ) and 24% ( $17.7 \text{ pg h}^{-1}$ ), respectively. This pattern was similar to that observed  
449 for NaDONA.

450 Particle deposition in the respiratory tract is primarily governed by inertial impaction, gravitational sedimentation, and  
451 Brownian diffusion, each dominating in different regions depending on particle size and airflow. Coarse particles deposit  
452 mainly in the upper airways via impaction and sedimentation, while ultrafine particles reach the distal pulmonary region  
453 through diffusion. Modelled deposition efficiencies by size and region are provided in the SI. Comparing the relative  
454 contribution to the deposition flux of the inhaled PFAS associated with the different size fractions, coarse particles showed the  
455 highest deposition flux in the head region ( $60\% \pm 3\%$ ), with lower contributions to the pulmonary ( $15\% \pm 2\%$ ) and  
456 tracheobronchial ( $3\% \pm 8\%$ ) regions. Fine particles ( $0.1\text{--}2.5 \mu\text{m}$ ), which dominate the PFAS size distribution, had the lowest  
457 overall deposition efficiency but were more evenly distributed:  $39\% \pm 10\%$  to the pulmonary region,  $25\% \pm 7\%$  to TB, and  
458  $18\% \pm 5\%$  to the head. Ultrafine particles shifted deposition toward the pulmonary region ( $45\% \pm 14\%$ ), with  $35\% \pm 11\%$  to  
459 TB and only  $8\% \pm 3\%$  to the head.

460 The modelled deposition behaviour of PFAS compounds investigated is closely linked to their particle size distribution. PFOS,  
461 GenX, 6:2 FTS, and PFBA exhibited significant ultrafine fractions, suggesting a higher likelihood of deep lung penetration.  
462 This is particularly concerning given that clearance mechanisms in the pulmonary region are slower compared to upper  
463 airways, and pulmonary deposition increases the potential for translocation into the bloodstream. These findings highlight the  
464 importance of considering both particle size and regional deposition when assessing inhalation exposure risks, especially for  
465 compounds with known toxicological profiles and environmental persistence. The modelling is not intended to represent  
466 population-level exposure or the full range of environmental conditions, but rather to evaluate size-resolved respiratory  
467 deposition behaviour for the measured aerosol populations.

#### 468 4 Conclusions

469 This study examined the aerosol formation and size-resolved distribution of a range of PFAS under controlled chamber  
470 conditions, using mixed water–organic systems with and without the model bacterium *Pseudomonas fluorescens* to assess the  
471 influence of molecular structure, interfacial behaviour and biological material on aerosol properties.

472 In terms of aerosolisation efficiency, sulfonated PFAS exhibited broadly similar aerosol-phase concentrations across chain  
473 lengths, whereas perfluoroalkyl carboxylic acids showed increasing aerosolisation with increasing chain length, highlighting  
474 the influence of functional group and hydrophobicity on the overall transfer of PFAS into the particle phase.

475 Most PFAS were associated with the fine aerosol mode, displaying unimodal mass–size distributions centred near 0.3  $\mu\text{m}$ .  
476 This consistent fine-mode behaviour across PFAS of differing chain lengths and functional groups indicates that aerosol  
477 formation was governed primarily by physical processes of droplet generation and evaporation. PFOS showed enhanced  
478 ultrafine enrichment, while 6:2 FTS and NaDONA displayed broader profiles, suggesting that differences in volatility and  
479 interfacial behaviour introduce secondary compound-specific effects.

480 The presence of *Pseudomonas fluorescens* as an aerosol seed did not enhance PFAS aerosolisation or alter modal diameters,  
481 but resulted in small, compound-specific reductions, particularly for PFEESA and GenX. The absence of PFAS enrichment at  
482 the bacterial modal diameter ( $\sim 0.6 \mu\text{m}$ ) indicates limited association of PFAS with bacterial surfaces under the tested  
483 conditions, likely reflecting electrostatic repulsion and preferential stabilisation of PFAS at air–liquid interfaces. These results  
484 suggest that biological material exerts only a minor influence on PFAS partitioning through the airborne pathway examined  
485 here; however, aqueous-phase sorption or complexation before aerosolisation may still contribute to water-to-air transfer and  
486 warrants further investigation. Moreover, if similar behaviour holds in the atmosphere, surface-active PFAS may avoid shifting  
487 into bioaerosol particle sizes with higher deposition velocities and therefore remain in the fine aerosol range with longer  
488 atmospheric lifetimes and transport potential.

489 MPPD simulations using the experimental size distributions indicated that most aerosol-bound PFAS would deposit in the  
490 pulmonary region. Compounds with stronger ultrafine enrichment, including PFOS, 6:2 FTS and GenX, showed higher  
491 predicted deposition in distal lung regions where clearance is slow and transfer into epithelial lining fluids is more likely.  
492 Under the studied conditions, PFAS-containing aerosols therefore fall largely within respirable size ranges relevant to  
493 inhalation exposure. It must be noted that MPPD modelling serves to contextualise size-resolved respiratory deposition for the  
494 observed aerosol populations, not to represent population-level exposure or comprehensive environmental scenarios. However,  
495 our results can be used as reference points for future field measurements and for evaluating the relevance of different aerosol  
496 generation processes that produce similar particle size ranges.

497 Overall, PFAS aerosolisation and particle-phase behaviour in the mixed solvent system were dominated by the physical  
498 processes of droplet formation and evaporation, suggesting that engineering and operational measures that suppress fine droplet  
499 production could reduce airborne PFAS emissions. The observed fine-mode distributions also imply that aerosolised PFAS  
500 may be efficiently transported in the atmosphere and contribute to inhalation exposure beyond immediate emission sources.

501 Although the water–methanol system does not fully reflect environmental conditions, it provides a controlled basis for  
502 identifying the fundamental processes governing PFAS transfer from contaminated aqueous systems to air. Variability in  
503 temperature and relative humidity may influence PFAS aerosol behaviour through effects on aerosol water content and  
504 evaporation dynamics and should be considered when extrapolating these findings to broader atmospheric conditions.  
505 Future work should apply this framework to more environmentally representative matrices, including natural organic matter,  
506 and diverse microbial assemblages, to better capture real emission complexity. Combining controlled chamber experiments  
507 with field measurements of size-resolved PFAS and bioaerosol emissions will be essential for improving predictions of PFAS  
508 atmospheric transport, deposition and human exposure.

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## 515 **6 Competing Interests**

516 Some authors are members of the editorial board of journal ACP. The authors have no other competing interests to declare.

## 517 **7 Author Contributions**

518 IK, SC, FM, EG, DM, PP conceived the study. IK, JPK, SC, FM, DM, EG, VV performed lab measurements, sampling, and  
519 sample analysis. IK, JPK, FM, EG performed data processing and interpretation. AB performed modelling. IK, JPK, FM, AB  
520 prepared the original draft of the paper. All authors contributed to reviewing and editing the manuscript.

## 521 **8 Data Availability**

522 The dataset for this work can be accessed at DOI [10.5281/zenodo.17756209](https://doi.org/10.5281/zenodo.17756209)

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