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Occurrence of per- and polyfluoroalkyl substances, pesticides, pharmaceuticals, and heavy metals in Greek backyard chicken eggs and estimation of the consumption risk

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Abbreviations and acronyms: ADONA, 4,8-dioxa-3H-perfluorononanoate; BQL, below quantification limit; LB, lower bound; CDPOS (Capstone B), 1-propanaminium, N-(carboxymethyl)-*N,N*-dimethyl-3-[[(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)sulfonyl]amino]-, hydroxide, inner salt; DEET, *N,N*-diethyl-m-toluamide; DPOSA (Capstone A), *N*-[3-(dimethyloxidoamino)propyl]-3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-1-octanesulfonamide; EFSA, European Food Safety Authority; EWI, estimated weekly intake; FASAs, perfluoroalkanelsulfonamides; FCAs, polyfluoroalkyl saturated carboxylic acid; FDEA, perfluorodecyl ethanoic acid; FDUEA, 2H-perfluoro-2-dodecenoic acid; FHEA, Perfluorohexyl ethanoic acid; FHUEA, 2H-perfluoro-2-octenoic acid; FOEA, perfluorooctyl ethanoic acid; FOUEA, 2H-perfluoro-2-decenoic acid; FTS, polyfluoronate saturated alkylsulfonic acid; FTUCAs, fluorotelomer unsaturated carboxylic acids; FUCAs, polyfluoroalkyl unsaturated carboxylic acid; GCB, graphitized carbon black; GenX or HFPO-DA, hexafluoropropylene oxide dimer acid; ICP-OES, inductively coupled plasma optical emission spectroscopy; IS, internal standards; LC-MS/MS, liquid chromatography tandem mass spectrometry; LC-QTOF-MS, liquid chromatography coupled to quadrupole time-of-flight mass spectrometry; LODs, limit of detections; LOQs, limit of quantifications; M8PFOS, perfluoro-1-[1,2,3,4,5,6,7,8-13C8]octanesulfonic acid; M8PFOS, mass-labelled perfluorooctane sulfonic acid; ML, maximum limits; N-EtFOSA, N-ethylperfluorooctanesulfonamide; N-EtFOSAA, 2-(N-ethylperfluorooctanesulfonamido)acetic acid; N-MeFOSA, N-methylperfluorooctanesulfonamide; N-MeFOSAA, 2-(N-methylperfluorooctanesulfonamido)acetic acid; PCA, principal component analysis; PCP, pentachlorophenol; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFBA, perfluorobutanoic acid; PFBS, perfluorobutanesulfonic acid; PFCAs, perfluoroalkyl carboxylic acid; PFDA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFDoS, perfluorododecanesulfonic acid; PFDPA, perfluorodecylphosphonic acid; PFDS, perfluorodecanesulfonic acid; PFECAs, polyfluoroethercarboxylic acid; PFESAs, polyfluoroethersulfonic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptanesulfonic acid; PFHxA, perfluorohexanoic acid; PFHxDA, perfluorohexadecanoic acid; PFHxPA, perfluorohexylphosphonic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFNS, perfluorononanesulfonic acid; PFOA, perfluorooctanoic acid; PFODA, perfluorooctadecanoic acid; PFOPA, perfluorooctylphosphonic acid; PFOS, perfluorooctanesulfonic acid; PFOSA, perfluoroctanesulfonamide; PFPAs, perfluoro alkyl phosphonic acid; PFPeA, perfluoropentanoic acid; PFPeS, perfluoropentanesulfonic acid; PFPrA, perfluoropropionic acid; PFSAs, perfluoroalkyl sulfonic acid; PFTeDA, perfluorotetradecanoic acid; PFTrDA, perfluorotridecanoic acid; PFUdA, perfluoroundecanoic acid; PSA, primary-secondary amine; PTFE, polytetrafluoroethylene; TIMS-QTOF-MS, hybrid quadrupole-trapped ion mobility spectrometry-time of flight mass spectrometer; TWI, tolerable weekly intake; UPLC-MS, ultra-performance liquid chromatography mass spectrometry; ∑4PFAS, sum of PFOS, PFOA, PFNA and PFHxS; 11Cl-PF3OUDS (F53-Minor), 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid; 4:2FTS, 1H,1H,2H,2H-perfluorohexanesulfonic acid; 6:2FTS, 1H,1H,2H,2H-perfluorooctanesulfonic acid; 6-Cl-PFHxPA, 6-chloroperfluorohexylphosphonic acid; 8:2FTS, 1H,1H,2H,2H-perfluorodecanesulfonic acid; 9ClPF3ONS (F53-Major), 9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid.

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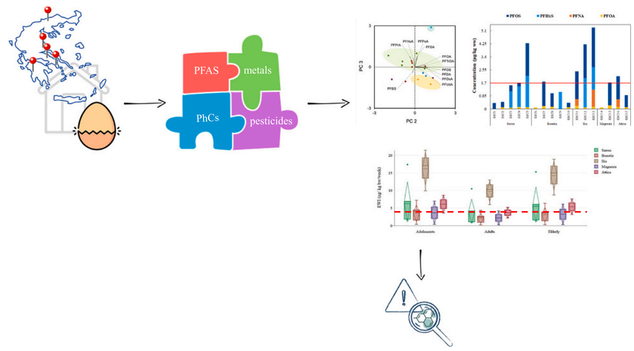
HIGHLIGHTS

- Backyard eggs from Greece analyzed for PFAS, metals and other micropollutants
- 17 to 24 PFAS found in each sample; higher concentrations for PFOS, N-EtFOSA, PFHxS
- Dinoterb and DEET detected in all samples, oxolinic acid was the predominant drug.
- A mean weekly intake of 7.05 ng/kg bw was calculated for PFAS in adolescents.
- The risk from eggs consumption depends on age group and studied area.

GRAPHICAL ABSTRACT



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ABSTRACT

Backyard chicken eggs are widely consumed worldwide, while they are also used as pollution bio-indicators for different groups of chemicals. In this study, home-produced eggs were collected from 17 chicken coops around Greece and analyzed for 46 per- and polyfluoroalkyl substances (PFAS), heavy metals, pesticide, and pharmaceutical residues. The number of PFAS detected per sample ranged from 17 to 24, while the mean concentration of Σ_{46}PFAS was 7.67 μg/kg ww. Perfluoroalkyl carboxylic acids contributed to 43 % of the total concentration, followed by perfluoroalkyl sulfonic acids (26 %) and perfluoroalkanelsulfonamides (16 %). Polyfluoroethercarboxylic acids, polyfluoroethersulfonic acids, and perfluoro alkyl phosphonic acids were detected occasionally and at low concentrations. Concerning the occurrence of individual PFAS, the highest mean concentrations were detected for PFOS (0.996 μg/kg ww), PFHxS (0.799 μg/kg ww), and PFBA (0.742 μg/kg ww). LC-QTOF-MS analysis indicated the co-presence of 6 pesticides and 5 pharmaceuticals in collected samples. Among pesticides, dinoterb and N,N-diethyl-m-toluamide (DEET) were found in all samples. DEET had the highest average concentration (8.25 μg/kg ww). Oxolinic acid was the most often detected pharmaceutical, with an average concentration of 1.03 μg/kg ww. The concentrations of heavy metals were low, and did not pose a threat to public health. In contrast, significant health concerns were raised due to the presence of PFAS, only 8 out of 17 samples were compliant to the limit set by the EU for eggs, while the mean PFAS weekly intake through eggs for adolescents and elderly exceeded EFSA's tolerable weekly intake.

1. Introduction

Chicken eggs are an essential component of diets worldwide, offering low-cost, nutrient-rich food that is either consumed on its own or incorporated into other cooked or processed products. The annual world consumption is estimated to be 150 eggs/year and per capita, while the corresponding value in Europe is 217 eggs, with notable differences among countries (Gautron et al., 2022). Besides the fact that the production methods have changed drastically during the last 50 years and most eggs in the EU are from commercial poultry farms (Eurostat, 2022), the production of backyard eggs for self-consumption and microtrade still remains a common practice in rural and semi-rural areas. In the U.S.A., a survey by the Department of Agriculture also indicated that in four U.S. metro areas (Denver, Los Angeles, New York and Miami), 0.8 % of all households owned chickens (USDA, 2012).

On the other hand, free-range eggs are widely used as environmental pollution bio-indicators and as identifiers of contaminated sites by different groups of chemicals (Zergui and Nkontcheu Kenko, 2024; Azam et al., 2024). The ingestion of contaminated soil particles by the hens, and the consumption of polluted drinking water and feed are some of the main routes contributing to elevated concentrations of specific chemicals in the eggs, such as dioxins and polychlorinated biphenyls (Petrlik et al., 2022), flame retardants (Haarr et al., 2023), pesticide residues (Alaboudi et al., 2019), and heavy metals (Aendo et al., 2022).

Per- and polyfluoroalkyl substances (PFAS) are defined as "*fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it)*" (OECD, 2021). So far, over 4700 CAS numbers of PFAS have been recorded (OECD/UNEP, 2018), while more than 200 categories of PFAS uses have been reported in different industry branches (Glüge et al., 2020). Given the widespread use of these compounds worldwide, various PFAS have been found in drinking water (Thomaidi et al., 2020) and soil (Arvaniti et al., 2024; Heimstad et al., 2024), posing a potential threat to human health due to their toxicological properties. In addition to the above, a 2023 journalistic investigation called "The Forever Pollution Project" (https://foreverpollution.eu/) identified nearly 23,000 sites in Europe contaminated by PFAS, while approximately 21,500 presumptive contamination sites were suggested based on current or past activities conducted at these locations (Cordner et al., 2024). The analysis of home-made eggs could potentially indicate the existence of contaminated sites by PFAS. However, to date, limited information is available on PFAS concentration levels in home-produced eggs globally. The existing data primarily come from three countries: Italy (Gazzotti et al., 2021; Stecconi et al., 2024), Belgium (Van Overmeire et al., 2009; Lasters et al., 2022, 2024), and China (Bao et al., 2019; Su et al., 2017; Qi et al., 2019). An earlier study also reported some data from the Netherlands and Greece (Zafeiraki et al., 2016). In some cases, samples were collected exclusively from areas near well-known point sources, such as fluorochemical plants (Su et al., 2017; Bao et al., 2019; Lasters et al., 2022, 2024), while in other instances, specific rural areas (Stecconi et al., 2024) or industrialized zones were selected (Qi et al., 2019). The number of PFAS analyzed ranged between 2 (Van Overmeire et al., 2009) to 29 analytes (Lasters et al., 2024), with most studies (7 out of 10 papers) analyzing between 10 and 20 substances.

In addition to environmental interest, there is also concern about the role of egg consumption in daily exposure to PFAS. In 2022, the European Food Safety Authority (EFSA) set maximum limits for four PFAS (PFOS, PFOA, PFNA, PFHxS) and their sum in eggs (European Commission, 2023). Furthermore, in an effort to expand knowledge on PFAS in food, the EU issued Recommendation 2022/1431, asking members states to monitor the presence of 22 well-known PFAS, as well as new generation PFAS, fluorotelomer alcohols, and sulfonates in various food products (European Commission, 2022).

Based on the above, we hypothesized that backyard eggs in certain areas of Greece—particularly those near industrial or urban environments—would contain elevated levels of PFAS compared to eggs from agricultural areas. Furthermore, we expected that the detected PFAS concentrations in some samples might exceed current EU limits, potentially leading to estimated dietary intakes above the tolerable thresholds set by EFSA. The main objectives of this study were to examine the occurrence of PFAS in home-produced eggs collected from various areas of Greece, to identify potentially contaminated regions and to estimate the probable risk of PFAS intake through egg consumption. For this reason, samples were collected from 17 chicken coops located in agricultural, industrialized, and urban areas and they were analyzed for the presence of 46 different substances using LC-MS/MS. The concentrations of PFAS were compared with the limits set by the EU, and the Estimated Weekly Intake (EWI) was calculated for different age groups. Collected samples were also analyzed for heavy metals using ICP-OES, while LC-QTOF-MS analysis was also applied to investigate the presence of other organic micropollutants such as medicinal products and pesticide residues. To the best of our knowledge, limited data is available on the occurrence of other emerging micropollutants in chicken eggs. The simultaneous determination of PFAS, heavy metals, and other organic micropollutants in the same samples provides valuable information on the various compounds and substances accumulated in backyard eggs and supports the prioritization of future monitoring campaigns.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents used in the current study are shown in Section 1 of Supplementary Material.

2.2. Sample design and collection

Between April and October 2023, 75 home-produced chicken eggs were collected from 17 chicken coops with the assistance of volunteers who raised free-ranging laying hens in 5 different prefectures across Greece: Serres (n = 5), Magnesia (n = 2), Boeotia (n = 5), Attica (n = 2), and Ilia (n = 3). All eggs from each chicken coop were sampled on the same day to ensure they came from different individual hens. The target areas include one highly urbanized region (Attica Prefecture), two highly industrialized regions (Magnesia and Boeotia), and two rural areas (Serres and Ilia) (Fig. S1). It is worth mentioning that the rural area of Ilia is also close to an airport. Four or five whole eggs from each household were lyophilized using a Labconco FreeZone 4.5 laboratory apparatus and homogenized to prepare 17 pooled samples (one per chicken coop). The collected samples contained approximately 75 % water content and were stored at −80 °C until analysis.

2.3. Analytical methodology

The methodology used for PFAS pretreatment and analysis as well as the analytical performance details are included in Supplementary Material (Section 2, Table S2). In brief, 1.0 ± 0.1 g of homogenized lyophilised egg sample was weighed into a 50 mL centrifuge tube, after pretreatment, the extracts were injected into an Exion UPLC system coupled to QTRAP mass spectrometer 6500+ (AB SCIEX, Canada). PFAS detection was based on the retention time and the ion ratio of the transition ions (±30 %) in relation with a standard solution mix according to the Guidance Document of PFAS produced by EURL-POPs version 2.0 (https://eurl-pops.eu/working-groups#_pfas).

For the analysis of pesticide and medicinal product residues, the modified QuEChERS method published by Drakopoulou et al. (2024) was used. The analysis of the final egg extracts was conducted using an ultra-high-performance liquid chromatographic (UHPLC) system (Elute 2, Bruker Daltonics, Bremen, Germany) coupled to a hybrid quadrupole-trapped ion mobility spectrometry-time of flight mass spectrometer TIMS-QTOF-MS (timsTOF Pro 2, Bruker Daltonics, Bremen, Germany). A dataset of these micropollutants and their transformation products is included in the wide-scope target database of the National and Kapodistrian University of Athens (doi:https://doi.org/10.5281/zenodo.13862245). The pretreatment method and the instrumental analysis parameters are provided in detail in the Supporting Material (Section 3). Overview of the schematic workflows followed for sample preparation are depicted in Figs. S2 and S3.

Egg samples were analyzed for element content after the microwave-assisted digestion method by using an Agilent 5800 VDV ICP-OES system. The applied method is described in the Supporting Material (Section 4).

Overall, for the quantification experiments, a pooled sample, composed of the less contaminated individual samples, in terms of the number of the detected compounds and the concentration levels of the detected chemicals, was used. Compound-specific LODs/LOQs for the detected contaminants, are provided in detail in Supplementary Materials (Table S4).

2.4. Health risk assessment

To assess the health risk associated with the consumption of PFAS contaminated eggs, the estimated weekly intake (EWI) was calculated using the following Eq. (1):

$$\mathrm{EWI}\,(\mathrm{ng/kg\,bw\,per\,week}) = \mathrm{C}_{\Sigma 4\mathrm{PFAS}}\,(\mathrm{ng/g}) \times \mathrm{Consumption}\,(\mathrm{g/kg\,bw/week}) \quad (1)$$

where, $C_{\Sigma 4PFAS}$ represents the average sum concentration of $\sum_4$PFAS included in EU legislation i.e., PFOA, PFNA, PFHxS, and PFOS in home-produced eggs (ng/g ww). Consumption data is the weekly egg consumption for Greek consumers (adolescents, adults, or elderly), as reported by the EFSA Comprehensive Food Consumption Database (EFSA, 2022).

EWI was compared to the established tolerable weekly intake (TWI) values for the sum of PFHxS, PFOS, PFOA, and PFNA (TWI: 4.4 ng/kg bw per week) (EFSA, 2018) and with the maximum tolerable risk (MTR) values for PFOS and PFOA (MTR: 43.8 ng/kg bw/week for PFOS and 87.5 ng/kg bw/week for PFOA) (Zeilmaker et al., 2016; Costopoulou et al., 2022).

Similarly, the weekly intake of heavy metals was calculated and compared to the available regulative values set by the EFSA (see Section 3.4).

2.5. Statistical analysis

The environmental contaminants detected < LOQ were reported as BQL (below the quantification limit). For the descriptive statistics and statistical treatment of the results, BQL values were replaced by a value equal to LOQ/2, following the QA/QC Directive (2009/90/EC). Similarly, a value equal to LOD/2 was used for those compounds measured < LOD.

When assessing the sample compliance to the legal limits set by EU legislation as well as for the health risk assessment, a different approach was conducted setting BQL values equal to zero (lower bound level, LB, according to EU Regulation 2023/915). The mean values of Σ_4PFAS across geographical areas were compared using one-way ANOVA, followed by the Duncan's post-hoc test. All tests were performed at the 0.05 significance level. Statistical analysis was performed using SPSS version 28.

Principal component analysis (PCA) with Varimax rotation was performed to examine potential relationships among PFAS occurrence and to identify any patterns in their geographical distribution. Further information on the use of PCA is provided in Supplementary Material (Section 5).

3. Results and discussion

3.1. *Per- and polyfluoroalkyl substances*

3.1.1. *Frequency of detection and occurrence of different classes*

Of the 46 target PFAS, 37 (80 %) compounds from 10 different classes were detected above the LOD in the 17 pooled home-produced egg samples collected from different chicken coops in Greece. All egg samples contained one or more PFAS, and the number of chemicals detected at concentrations higher than the LOD per sample ranged from 17 to 24. The results for all analytes, for each group of contaminants and per sample are reported in Table S5 and visualized as a heatmap in Fig. S4, while the PFAS profile and the concentrations of all detected compounds in the eggs, along with the statistical descriptors, are summarized in Table S6 (see also Figs. 1–2). Fig. 1 illustrates (a) the frequency of appearance (FoA) of the detected PFAS and (b) the mean concentration of detected PFAS in egg samples. Among 46 PFAS, 14 substances (PFPrA, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFDA, PFUdA, PFTrDA, PFTeDA, PFBS, PFHxS, PFOS, 6:2 FTS) were detected at 100 %, 23 substances were occasionally detected while the remaining 9 substances (PFPeS, PFDS, PFDoS, PFHxPA, FDUEA, FOUEA, FDEA, FOEA, GenX) were not detected in any sample.

The concentrations of 46 individual PFAS in home-produced egg samples varied by 3 orders of magnitude from <LOD to 2.57 μg/kg wet weight (ww), and the mean concentration measured in the present study for Σ_{46}PFAS was 7.67 μg/kg ww (Table S5). As regards the role of different classes, PFAS composition was primarily determined by PFCAs contributing an average of 43 % to the sum of 46 PFAS level (mean Σ_{14}PFCAs 3.40 μg/kg ww, range <LOD–2.49 μg/kg ww), followed by the Σ_8PFSAs with 26 % (mean 1.90 μg/kg ww, range <LOD–2.57 μg/kg ww) (Figs. 1b and 2, Table S5). The third highest contribution was from Σ_5FASAs (mean 1.21 μg/kg ww, range <LOD–1.92 μg/kg ww) that contributed to an average of 16 % mass of Σ_{46}PFAS (Figs. 1b and 2, Table S5). The remaining groups contributed less than 15 % to the total Σ_{46}PFAS mass; specifically, Σ_3FCAs 8 %, Σ_2Capstone 3 % and Σ_3FUCAs 2 %. PFECAs, PFESAs, FTS, and PFPAs were infrequently detected and at low concentrations, when present, collectively contributed less than 1.5 % to Σ_{46}PFAS (Figs. 1 and 2, Table S5).

3.1.2. *Occurrence of individual PFAS*

Concerning the occurrence of individual PFAS, relatively high concentrations of PFOS, N-EtFOSA, PFHxS, and PFBA were determined in egg samples (Fig. 1b, Tables S5 and S6). Specifically, among all targeted analytes, the highest concentrations were detected for PFOS (mean concentration 0.996 μg/kg ww). It is worth mentioning that PFOS contributed 46 % to Σ_8PFSAs and 13 % to the total PFAS profile, determined at the highest levels of the PFSAs group at 9 out of 17 eggs collected from backyard chickens (EBC), while its highest concentrations were found in egg samples from Ilia Prefecture (EBC13: 2.57 μg/kg ww; EBC12: 2.21 μg/kg ww). As regards N-EtFOSA, it was detected in 94 % of the samples (values between LOD and LOQ for 14 out of 17 samples), while the highest measured concentrations were 1.92 and 1.84 μg/kg ww in egg samples from EBC11 and EBC12, respectively. PFHxS contributed, on average, 10 % to all PFAS analytes, with a mean concentration of 0.799 μg/kg ww, while its highest concentration was determined in eggs from EBC5 (2.05 μg/kg). PFBA was the dominant of all fourteen perfluoroalkyl carboxylic acids in ten egg samples, with a mean concentration of 0.742 μg/kg ww, contributing 22 % to all PFCAs analytes on average. Its highest concentration was found in eggs from

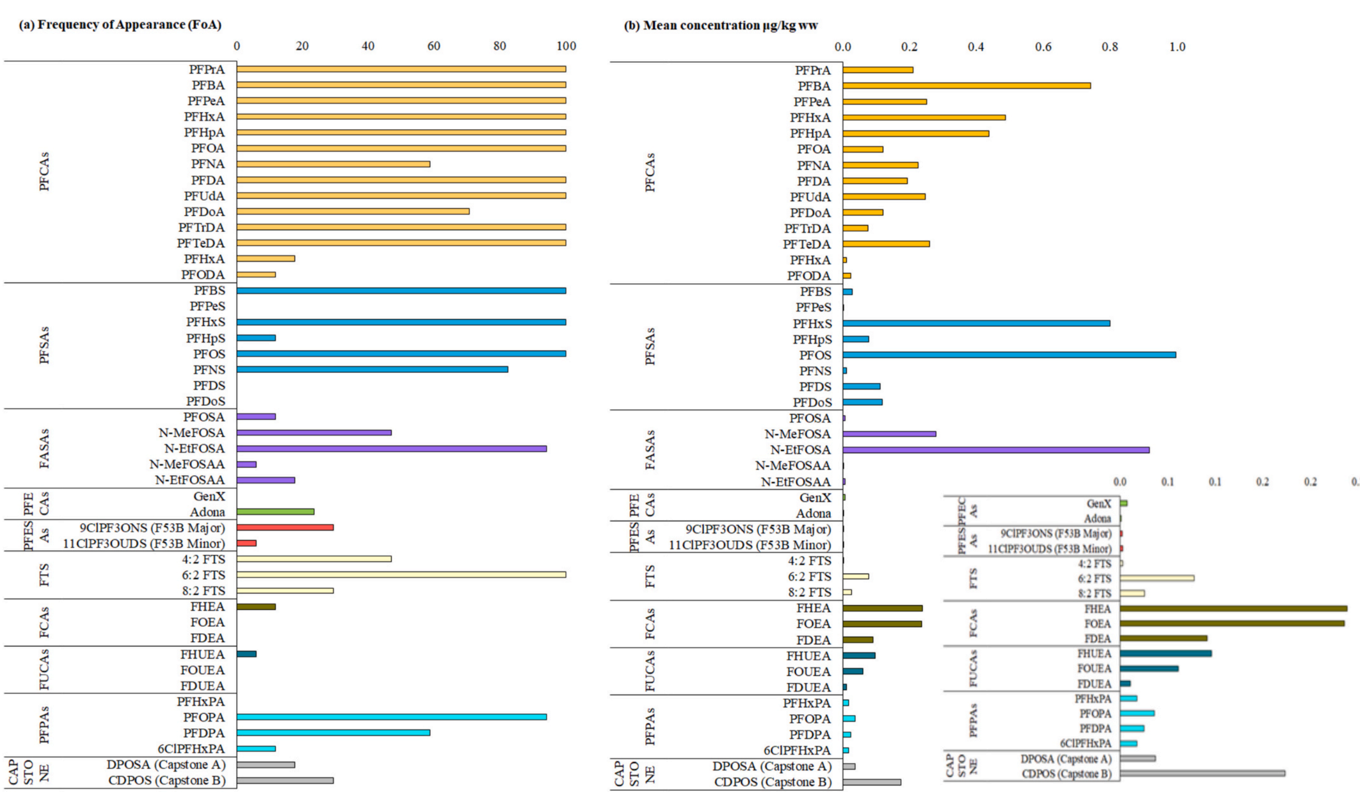


Fig. 1. (a) Frequency of appearance and (b) mean concentrations (μg/kg ww) of studied PFAS from 17 Greek home-produced egg samples.

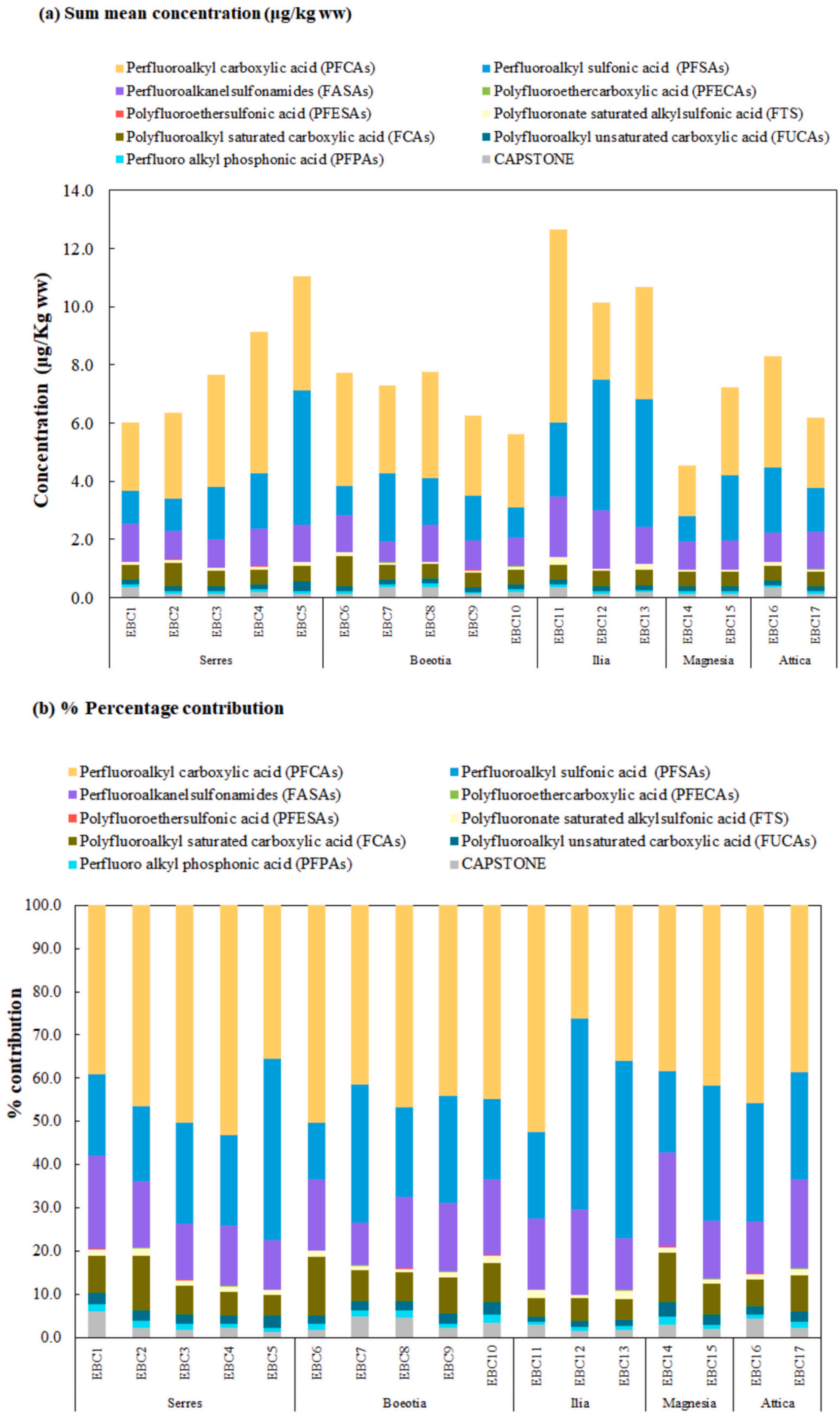


Fig. 2. Contribution of different classes of PFAS to the sum concentrations measured in home-produced eggs collected from backyard chickens (EBC) in different Greek regions.

EBC11 (2.49 μg/kg ww). Although PFOA was detected in all samples with a mean concentration of 0.120 μg/kg ww and a range of 0.0396 (EBC1 and EBC9) to 0.226 (EBC15) μg/kg ww, it was not the most abundant PFCA. Overall, the short-chain PFCAs (C_{3-7}) concentrations were higher in homegrown eggs (mean ∑short-chain PFCAs 2.13 μg/kg ww on average), as opposed to long-chain $C_{8-14,16,18}$ carboxylates (mean ∑long-chain PFCAs 1.28 μg/kg ww).

Previous studies have mainly focused on legacy PFAS contamination, such as PFCAs and PFSAs in home-produced eggs, and somewhat less attention has been paid to other fluorochemicals. In the current study, we also focused on eight additional groups of emerging and precursors PFASs, as shown below.

Five FASAs, including PFOSA, *N*-MeFOSA, *N*-EtFOSA, *N*-MeFOSAA, and *N*-EtFOSAA, were analyzed in 17 eggs for the first time. Results showed that *N*-MeFOSA (mean concentration 0.278 μg/kg ww, range 0.143–0.430 μg/kg ww) was the dominant FASAs compound after *N*-EtFOSA, while only trace amounts of PFOSA, *N*-MeFOSAA, and *N*-EtFOSAA were found in most egg samples. One significant class of replacement chemicals is fluorinated ether compounds consisting of short-chain perfluorinated carbons series linked by ether oxygens and capped with an acidic head group. These alternatives include PFECAs and PFESAs, which are considered to have lower environmental persistence and bioaccumulation, yet little is known about the potential toxic effects of these emerging contaminants (McCord et al., 2018; Lu et al., 2019; Hu et al., 2024). In this study, two PFECAs, hexafluoropropylene oxide dimer acid (HFPO-DA, commercial name GenX) and 3*H*-perfluoro-3-[(3-methoxy-propoxy)propanoic acid] (ADONA), polyfluorinated alternatives to PFOA; and two PFESAs, 9ClPF3ONS (F53B Major) and 11ClPF3OUDS (F53B Minor), were monitored in egg samples. As regards new generation PFAS, GenX was not detected in any of the collected samples, ADONA was found in four samples (EBC2, 3, 4, 12) with concentrations up to 0.0147 μg/kg ww, while 11ClPF3OUDS and 9ClPF3ONS in one (EBC9) and five samples (EBC1, 3 7, 12, 16), respectively, at concentrations above LOQ (Tables S5 and S6).

Perfluoroalkyl phosphonic acids (PFPAs) are a sub-group of PFAS that have been commercialized since the 1970s, particularly as defoamers in pesticide formulations and wetting agents in consumer products (Wang et al., 2016). In this study, four PFPAs, including PFHxPA, PFOPA, PFDPA, and 6ClPFHxPA were monitored. Among these compounds, PFOPA and PFDPA were detected in 94 % and 59 % of the eggs examined, respectively, but with concentrations lower than the LOQ (Table S5). PFHxPA and 6ClPFHxPA were not detected in any of the samples (Table S5).

As regards fluorotelomer sulfonates, three compounds were detected in egg samples. 6:2 FTS was the most abundant contaminant, with the highest concentration (EBC5: 0.116 μg/kg ww) and a mean value of 0.078 μg/kg ww (Table S5). 8:2 FTS was detected in three samples above the LOQ while 4:2 FTS in eight samples (Table S5).

Two emerging substances, including Capstone A (DPOSA) and Capstone B (CDPOS) were also analyzed, as part of this study. Their levels in all samples were below the quantifiable limit. These precursor chemicals, which have not yet been incorporated in the guidelines of environmental laboratory standard lists, are polyfluorinated chemicals that degrade in the environment to persistent perfluorinated compounds (Cornelsen et al., 2021).

Fluorotelomer saturated and unsaturated carboxylic acids (FCAs and FUCAs) are intermediate compounds formed from the biotransformation of fluorotelomer-based compounds (Buck et al., 2011). As part of this study, three FCAs (FHEA, FOEA, and FDEA) were analyzed in the 17 egg samples. The only compound of this class above the LOQ was FHEA, which was detected in only two samples. As for FUCAs, only FHUEA was found in one sample at a concentration of 0.252 μg/kg ww (Table S5).

Comparison of the observed concentrations with those reported in the literature shows that the mean concentration of $\sum_{46}$PFAS was 7.67 μg/kg ww, which is higher than the 2.37 μg/kg ww reported in a previous Greek survey of home-produced egg yolks in 2016 (Zafeiraki et al., 2016). However, it is important to consider that i) a broader list of PFAS was targeted in this study (46 substances instead of 11), including emerging and precursors PFAS that were found in the μg/kg ww range, ii) the sampling sites in the two studies were different, and iii) the analysis in the current study was performed on the whole egg and not only in the yolk. As regards other countries, Zafeiraki et al. (2016) and Gazzotti et al. (2021) measured relatively low concentrations of ΣPFAS in home-produced eggs from the Netherlands and Italy, with mean sum PFAS concentrations of 4.84 (11 PFAS) and 1.28 (4 PFAS) μg/kg ww, respectively. So far, the highest reported concentration for the average sum of PFAS is 122 μg/kg (17 PFAS) in egg yolk samples collected nearby a Chinese PFAS industry (Wang et al., 2019), and 58.2 μg/kg ww (12 PFAS) in whole eggs collected from households surrounding a fluorochemical industrial park in China (Su et al., 2017). The concentrations of individual compounds found in the current study were similar or lower than PFAS levels detected in home-produced egg samples of other countries. Concerning PFOS, its concentrations align with those reported for eggs collected in Greece (range: <0.5–8.9 μg/kg μg/kg ww) (Zafeiraki et al., 2016) and Italy (range: 0.25–3.47) (Gazzotti et al., 2021), while they are considerably lower than those obtained from the Netherlands (range: <0.5–24.8 μg/kg μg/kg ww) (Zafeiraki et al., 2016), Belgium (range: 0.54–241 μg/kg ww) (Lasters et al., 2022) and China (range: <LOD-1062 μg/kg ww) (Su et al., 2017; Wang et al., 2019). PFBA has been detected at approximately 2–3 times higher levels in eggs from Belgium (0.40–9.1 μg/kg ww) (Lasters et al., 2022) compared to this study, while no data are available from other European countries. The levels of PFOA in the current study were similar to those reported in the past for Greece (<0.5–2.7 μg/kg ww) (Zafeiraki et al., 2016) and Belgium (0.13–2.4 μg/kg ww) (Lasters et al., 2022), higher than those of Italian eggs (Gazzotti et al., 2021) and lower than those determined in China, ranging up to 125 μg/kg ww (Su et al., 2017).

The exposure pathways responsible for the PFAS found in Greek egg samples were not studied in the current article, as the main objective was to investigate the occurrence of a broad range of targeted PFAS in home-produced eggs collected from different regions. However, according to the literature, the potential PFAS exposure pathways for hens living in chicken coops seem to be soil, water, and food. Organic and free-range poultry eat earthworms, which may absorb PFAS and pass it to hens. Given that breeding chickens can consume 2–60 g of soil with their food, the soil has also a significant impact on egg contamination. Plants growing on the paddocks where hens feed could also be a source, particularly for short-chains for which the transfer rates from the soil are higher (Mikolajczyk et al., 2022). In addition to the direct transfer of PFAS, their precursors transferred into hens' eggs can contribute to the overall PFAS burden (Mikolajczyk et al., 2022).

3.1.3. Principal component analysis

PCA has previously been used to identify PFAS association patterns, i.e. how the concentrations of different PFAS compounds varied together, and sources in various environmental settings and compartments, such as gull eggs (Colomer-Vidal et al., 2022), surface waters (Zhang et al., 2016), soil (Wellmitz et al., 2023) or human serum (Hu et al., 2018). Due to the small sample size, the PCA results for eggs from Greece should be interpreted with caution. Nonetheless, the identified clear patterns may be considered indicative of the distribution of PFAS in Greek eggs. The results are presented in Fig. S5.

The first three principal components (PCs) explain 72 % of the total variability in the data. The first PC, which accounts for 28 % of the variability, includes large loadings (>0.80) of 6:2 FTS, PFHpA, and PFTeDA, along with a moderate contribution of a mix of other compounds such as PFPeA, PFHxA, PFUdA, PFTrDA, and PFBS (loadings 0.42–0.67). The second PC captures an additional 28 % of the variability and has large loadings (0.73–0.93) for long-chain PFCAs, i.e. PFOA, PFDA, PFDoA, PFTrDA, and PFOS. The third PC (16 % of the variability) groups the short-chain C_3-C_6 PFCAs, with moderate to high loadings (0.59–0.79). The inclusion of PFPeA, PFHxA and PFTrDA in two PCs, though with varying importance, may reflect their multiple direct and indirect sources in the environment through which they may have found their way to the eggs. For instance, PFPeA and PFHxA apart from being associated with the other short-chain perfluoroalkyl carboxylates, are also associated with 6:2 FTS on PC1. This may reflect the aerobic biotransformation of 6:2 FTS to C_4–C_7 perfluoroalkyl carboxylates, which has been witnessed in soil (Yan et al., 2024) as well as in activated sludge (Wang et al., 2011), and landfill leachate (Méndez et al., 2022).

The projection of the sample scores onto the biplots of the PCs shows that all samples from Serres are clearly separated from the others based on positive values of PC1. Additionally, an outlying sample from Boeotia also has a large positive score on PC1 in contrast to the remaining Boeotia samples, which are characterized by low concentrations of the compounds strongly correlated with PC1. Samples from Ilia and one sample from Attica have positive scores on PC2 reflecting thus high occurrence of long-chain PFCAs and/or PFOS, whereas samples from Boeotia and Serres, with positive scores on PC3, appear to be enriched with short-chain compounds. PC3 also helps identify an outlier sample from Ilia in which the highest values of C_3–C_6 PFCAs were recorded.

3.2. Occurrence of other organic micropollutants

LC-QTOF-MS analysis showed that six pesticide residues (dinoterb, *N*,*N*-diethyl-m-toluamide (DEET), fipronil sulfone, pentachlorophenol (PCP), piperonylbutoxide and tricyclazole) were present in collected samples (Fig. 3, Tables S7 and S8, Fig. S6). Among them, dinoterb and DEET were found in all samples, and fipronil sulfone in 16 out of 17 samples, while the frequency of detection for the other three substances ranged from 6 to 12 %. The sum concentrations of pesticides ranged between 3.75 (EBC17) and 40.71 (EBC8) μg/kg ww, while the predominant compound for 15 out of 17 samples was DEET. This compound had also the highest average concentration (8.25 μg/kg ww). As regards the highest observed concentrations, they were measured for dinoterb (EBC8: 24.0 μg/kg ww), PCP (EBC4: 23.1 μg/kg ww) and DEET (EBC10: 20.4 μg/kg ww). In contrast, the concentrations of the other substances did not exceed 7.41 μg/kg ww (for fipronilsulfone in EBC5).

To the best of our knowledge, except for PCP, this is the first study that examines the occurrence of these organic micropollutants in hens' eggs. According to Pubchem (2024), dinoterb is used as herbicide and rodenticide, DEET is the primary ingredient of most insect repellents, while fipronil sulfone is the main transformation product of fipronil, a well-known insecticide. PCP was widely used in the past as a wide-spectrum pesticide and in several other applications such as wood preservative and it is considered a priority pollutant in the EU (European Commission, 2013). There are few papers that report its concentration in chicken eggs. In a recent article, Zhou et al. (2021) collected hen eggs from Chinese markets and supermarkets and reported an average concentration of 0.40 μg/kg ww. Fernandes et al. (2019) reported PCP average concentration of 0.54 μg/kg in eggs collected from the UK. In an older study, Frank et al. (1990) collected samples from Canada and reported much higher concentrations of PCP (average concentration: 35 μg/kg; maximum concentration: 160 μg/kg). On the other hand, in several previous studies, PCP is considered as one of the sources which are related to the detection of dioxins in chicken eggs (Harnly et al., 2000; Piskorska-Pliszczynska et al., 2016). Considering that in the current study PCP was detected in eggs originating from two specific chicken coops, future research should focus on these areas, while analyses of dioxins should be conducted in parallel.

Additionally, to biocides, five medicinal products were occasionally determined in collected samples (Fig. 3, Tables S9 and 10, Fig. S7). Ciprofloxacin, citalopram, its metabolite nor-citalopram, and fluconazole were detected in only one sample, whereas oxolinic acid was found in 6 out of 17 samples. Oxolinic acid is a quinolone antibiotic commonly used for veterinary drug in poultry and other animals. Its average concentration in the current study was equal to 1.03 μg/kg ww, while the highest concentration was 3.19 μg/kg ww (EBC4). The concentrations of other substances ranged between 7.44 (ciprofloxacin) and 48.6 μg/kg ww (nor-citalopram). To the best of our knowledge, it is the first study in which these pharmaceuticals are detected in egg samples.

3.3. Risk assessment and potential exposure of consumers from PFAS in home-produced eggs

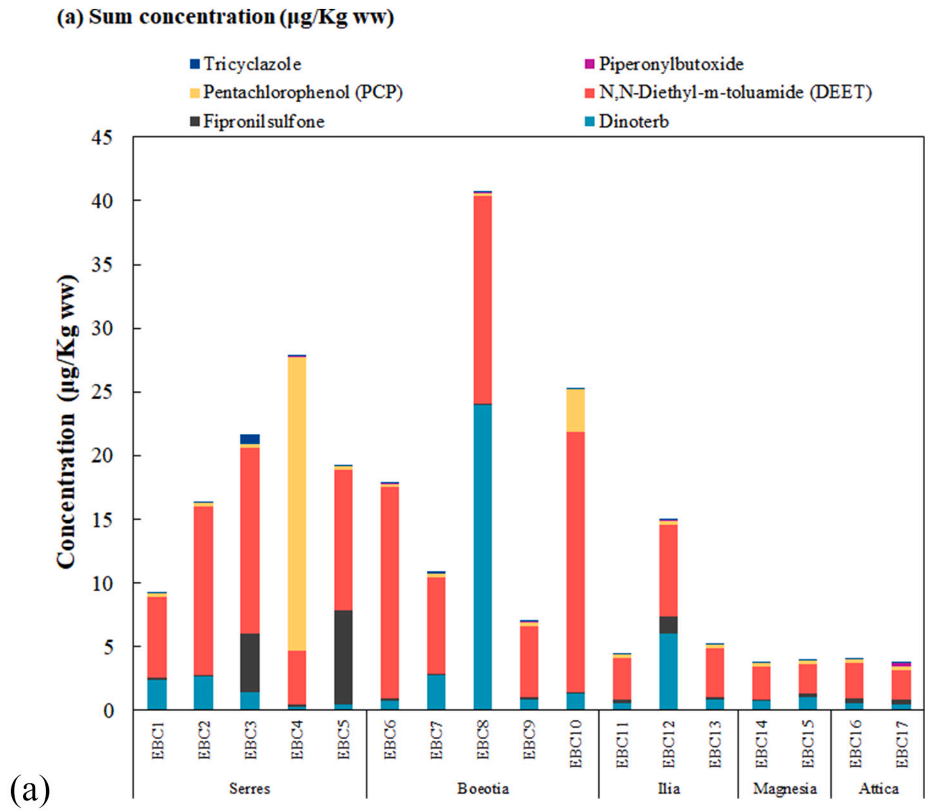
Public concern about PFAS increased after several studies indicated their presence in the environment, and in the human body (Squadrone et al., 2014). Toxicological evidence has associated certain PFAS with adverse effects on health, such as damage to the immune system and liver, birth defects, cancer, and delayed development (Sunderland et al., 2019; Fenton et al., 2021). Furthermore, epidemiological data found significant associations between PFAS exposure and adverse immune effects in children as well as dyslipidemia in adults (Rappazzo et al., 2017).

The European Regulation 2023/915 has set maximum limits (ML) in eggs equal to 0.30, 1.0, 0.30, and 0.70 μg/kg ww for PFHxS, PFOS, PFOA, and PFNA, respectively. For PFOA, the values determined in the egg samples of the present study were well below the ML set from the EU (Table S5). However, as regards PFOS, PFHxS, and PFNA, seven, six and one out of 17 samples, respectively, were above the ML (Table S5). Considering that the ML set by the EU for the sum of these four PFASs (Σ_4PFAS) in eggs is equal to 1.7 μg/kg ww (European Commission, 2023) and following the assumption, suggested by EU Regulation 2023/915, that all the values below the LOQ should be considered zero (LB level), only 9 out of 17 analyzed samples were compliant to this limit (Fig. 4a). For the samples that exceed surpass the maximum set level, Σ_4PFAS concentrations ranged from 1.71 (EBC4) to 5.30 (EBC13) μg/kg ww (Table S5). These results indicate the important contamination of home-produced eggs by regulated PFAS. The samples collected in the frame for this study, from different Greek regions, indicate that the contamination is highest in the Prefecture of Ilia.

Food consumption has been recognized as the most significant source of human exposure to PFAS for the general population (Costopoulou et al., 2022; Domingo et al., 2012). Since eggs are inevitably exposed to these bioaccumulative compounds in the natural environment, egg consumption may eventually cause adverse health impacts on humans (Domingo, 2014; Miranda et al., 2015; Lasters et al., 2022).

To estimate the risk deriving from PFAS contaminated eggs, the weekly intakes (EWI) of PFAS by egg consumption were also calculated using Eq. 1. According to the LB level calculations (best-case scenario), EWIs values for the combined exposures of PFOS, PFOA, PFNA, and PFHxS as a result of consumption of backyard eggs correspond to a mean weekly intake of 7.05, 4.26, and 6.20 ng/kg bw for adolescents, adults and elderly in Greece, respectively (Table S11 and Fig. 4b). These values for the age groups of adolescents and elderly exceed EFSA's TWI for food (4.4 ng/kg/w) and therefore raise significant health concerns for egg consumers. The numbers (%) of studied EBCs, in Greece, in which the EWI was above EFSA health guidelines, ranged between 29 % and 53 % depending on the age group (Table S11). The comparison of the relevant figures in different Greek areas shows important differences with the highest risk observed in the Prefecture of Ilia, where all EBCs exceeded EFSA health guidelines for all age groups. When the risk from the consumption of eggs was estimated exclusively for PFOS and PFOA, comparing the relevant EWI values with the maximum tolerable risk thresholds for these compounds, intakes proved to be comparatively low for all studied areas and age groups (Tables S11).

So far, there is limited information in the literature on exposure to PFAS through the consumption of eggs. In a recent study, Lasters et al. (2022) focused on a 10 km radius region around a fluorochemical plant in Belgium and found that the TWI of 4.4 ng/kg bw per week was exceeded in 67 % of the locations for all age groups. Costopoulou et al. (2022) estimated the PFAS intake from fish, eggs and water for the total population in Greece and reported that the mean weekly intake was above TWI mainly due to fish consumption. Eggs contributed almost 25



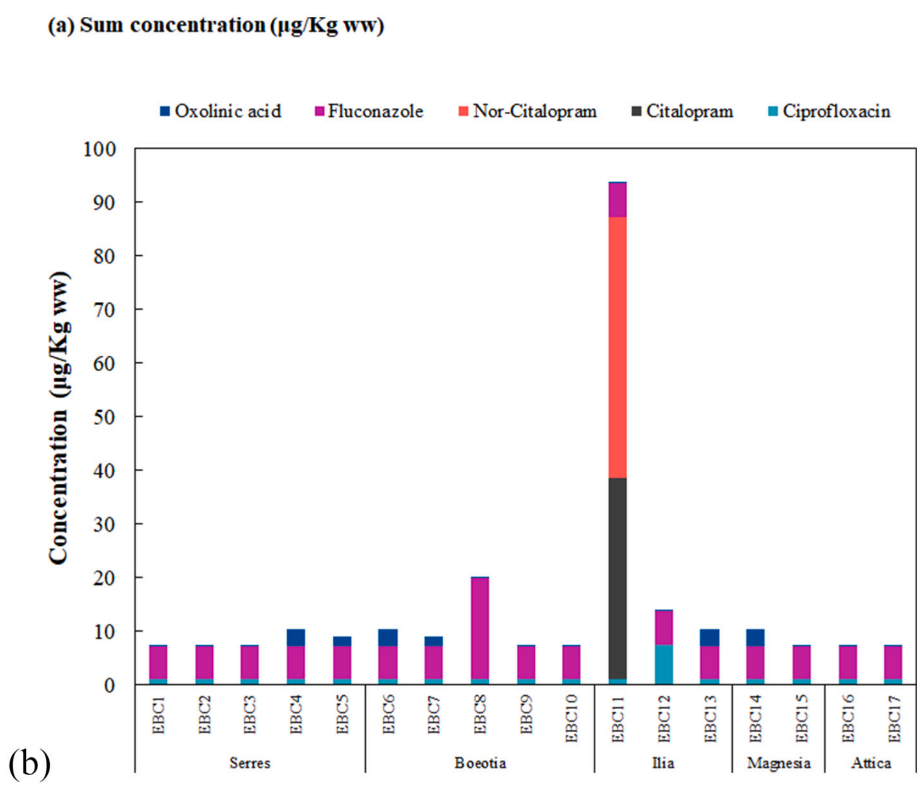


Fig. 3. Concentrations (as μg/kg ww) of pesticides and insecticide residues (a) and medicinal products and their transformation products (b) in Greek home-produced eggs collected from backyard chickens.

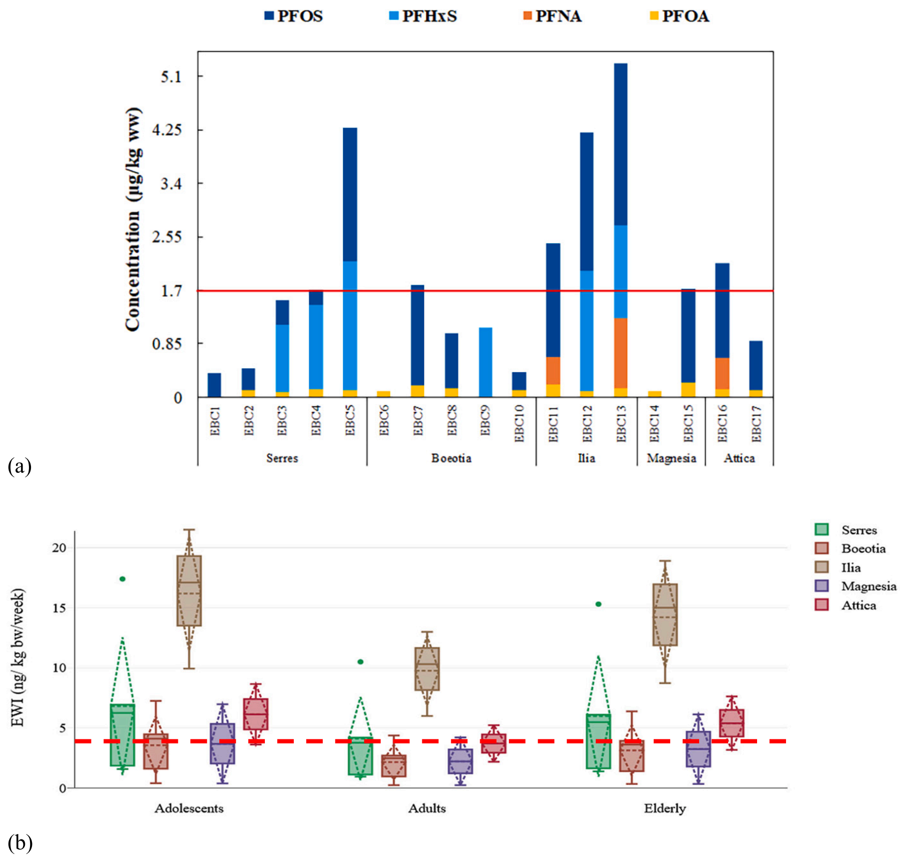


Fig. 4. Σ4PFAS (PFOS, PFHxS, PFNA and PFOA) concentrations in Greek home-produced eggs collected from different (a) and Estimated Weekly Intake (EWI) of Σ4PFAS through backyard eggs consumption for different age groups of Greek population and different Greek areas (b).The red line shows the limit set by EU legislation 2023/915 (a) and the tolerable weekly intake (TWI) set by EU legislation for the sum of these substances (b).

% to PFAS daily intake with 0.16 ng/kg bw/day (1.12 ng/kg bw/week). In that study, the risk assessment was based on earlier analyses of egg samples (Zafeiraki et al., 2016), while the collected eggs were a mixture of home-produced and commercial eggs. To the best of our knowledge, this is the first study that estimates the risk to human health for backyard eggs consumption at a country-level.

Previous studies have shown that home-produced eggs contain higher concentrations of PFAS compared to those from various types of commercial farming (Stecconi et al., 2024, and references therein), likely due to the ingestion of contaminated food sources (such as worms and insects) as well as soil ingestion during feeding (Zafeiraki et al., 2016; Gazzotti et al., 2021). In line with this finding, the Dutch National Institute for Public Health and the Environment (RIVM) recently advised against the consumption of home-produced eggs, as they contribute to the already significant PFAS intake from food and drinking water in the Netherlands (RIVM, 2025).Thus, the risk assessment based on our results applies to backyard egg consumers in Greece, rather than the general population that purchases commercially produced eggs. To our knowledge, backyard egg consumers are primarily rural residents or environmentally conscious urban individuals who deliberately rely on this source of eggs. According to the Greek Ministry of Rural Development & Food, 77.8 % of the annual egg production for consumption in 2023 is estimated to come from intensive (systematic) farming units, with the remaining share attributed to backyard (traditional) poultry farming. Consequently, the risk posed by PFAS through the consumption of home-produced eggs may affect approximately one-fifth of the Greek population.

3.4. Heavy metals and macroelements

Among all the heavy metals determined, the essential metals Fe, Zn, Cu, and Mn (Zoroddu et al., 2019) were the most abundant in the eggs, whereas the concentrations of all other metals were below the LOD in all samples, with the only exception being a sample from Attica, in which Pb was 0.26 mg/kg indicating a local contamination (Fig. S8, Tables S12 and 13). Although the European Commission has established maximum levels (MLs) for the most toxic metals, such as Pb, Cd, Hg, As, and Sn, in various food commodities, this set of regulatory limits does not include eggs. Therefore, the concentrations found in this survey were compared to values reported in the literature for both home-produced and commercially produced eggs (Table S13). The concentrations of the essential metals are in good agreement with the values found in previous studies. The higher concentrations of various metals found in eggs from various Brazilian cities, as well as of Cu in India, were attributed to metal-fortified feed and soil contamination (de Freitas et al., 2013; Giri and Singh, 2019). The essential metal content of Greek eggs is also in line with the mean content of hen eggs across seven European countries, which is 0.07, 20.2, and 13.4 mg/kg for Cu, Fe, and Zn, respectively (EFSA, 2024). The relatively narrow variance of these metals in eggs may reflect their homeostatic regulation in the hen's body, which, however, can be disrupted under high environmental stress, such as in mining areas with polymetallic pollution (Kribi-Boukhris et al., 2020).

The high LODs attained in ICP-OES, compared to those in ICP-MS, do not allow the direct comparative evaluation of As, Cd, and Co levels in eggs from Greece respect to studies from other countries in which more sensitive technique were employed. Nonetheless, the non-detected values of Cr, Ni, and Pb do not imply contamination of the samples with these metals.

The macroelement concentrations in the analyzed eggs were the follows: Ca: 430 ± 40 (361–484); K: 1316 ± 128 (1055–1659); Mg: 123 ± 15 (102–153); Na: 1154 ± 116 (910–1395); P; 1904 ± 101 (1717–2085) — all in mg/kg ww. These values are in good agreement with those reported for organic and non-organic eggs from Brazil when expressed in wet weight, although Greek eggs, on average, have higher Ca and P content (Borges et al., 2015). They also correspond to the mean composition of eggs within the EU, with mean concentrations of 571, 1313, 124, and 2071 mg/kg ww for Ca, K, Mg, and P, respectively, although they seem to contain less Ca and P than the eggs in other European countries (EFSA, 2024).

An estimate of the potential health risk from toxic metals in eggs from Greece was made by considering the LOQ as the maximum potential concentration, since they were below the detection limit of the analytical technique employed. The criteria used for comparison were the legislative critical values currently valid in the EU. Specifically, the Panel on Contaminants in the Food Chain (CONTAM Panel) of EFSA, based on dose-response data for As from key epidemiological studies, selected a benchmark dose lower confidence limit for a 1 % extra risk (BMDL01) between 0.3 and 8 μg/kg bw/day (EFSA, 2009), which corresponds to 2.1–24 μg/kg bw/week. They also suspended the tolerable weekly intake (TWI) of 25 μg/kg bw for Pb, which was valid until 2010, and estimated a BMDL01 for various lead-induced health effects, among which the most stringent is 12 μg/kg bw/day (84 μg/kg bw/week) (EFSA, 2010). As opposed to Pb, a tolerable weekly intake (TWI) for Cd of 2.5 μg/kg bw (EFSA, 2011) and a tolerable daily intake (TDI) of 13 μg/kg bw (91 μg/kg bw/week) are currently accepted in the EU (EFSA, 2020). As for Cr, the CONTAM Panel derived a TDI of 0.3 mg/kg bw per day (2100 μg/kg bw/week) for Cr(III) and assumed that all chromium in food is Cr(III). The maximum weekly exposure of adults in Greece to each of the above metals through the consumption of eggs is estimated to range from 0.18 to 0.59 μg/kg bw, which is far below the values mentioned above and, therefore, not capable of raising concern. This assumption is in accordance with the conclusions of a previous study on metals in eggs from poultry farms in northern Greece, where, although health risks were not assessed, the determined levels were far below the toxic limits reported in the literature (Nisianakis et al., 2009).

4. Conclusions

A possible threat to human health due to the occurrence of PFAS in backyard Greek eggs was found. Only 9 out of 17 samples were compliant to the limit of 1.7 μg/kg ww set by the EU for the sum of PFHxS, PFOS, PFOA, and PFNA. Calculation of the PFAS EWI through egg consumption showed that the risk differs according to the age group and studied area, with the highest risks estimated for adolescents and the Prefecture of Ilia. Taking into account that, according to EFSA, the TWI is currently based on the occurrence of 4 PFAS (PFHxS, PFOS, PFOA, and PFNA) while the number of PFAS found in each egg sample ranged between 17 and 24, an underestimation of the possible risk to human health cannot be excluded. The heavy metal and macroelements concentrations in the examined eggs were consistent with values reported in the literature, though generally lower than in eggs collected from heavily polluted areas. In any case, the potentially toxic metal content does not pose a risk to public health. For the first time, pharmaceuticals and pesticide residues were found in backyard chicken eggs. The systematic presence of most of them is related to commonly applied agricultural practices. Further research should focus on the exposure pathways responsible for the PFAS found in Greek backyard egg samples.

CRediT authorship contribution statement

Olga S. Arvaniti: Writing – original draft, Visualization, Project administration, Methodology. **Dimitrios Triantafyllos Gerokonstantis:** Writing – original draft, Investigation. **Christoforos Bouzoukas:** Writing – original draft, Investigation. **Maria Aloupi:** Writing – original draft, Validation, Investigation, Formal analysis. **Georgios Gkotsis:** Writing – original draft, Visualization, Software, Investigation. **Antigoni Konomi:** Writing – original draft, Investigation. **Artemis Mastrotheodoraki:** Writing – original draft, Investigation. **Athanasia Iliopoulou:** Writing – original draft, Investigation. **Marios Kostakis:** Writing – review & editing, Visualization, Validation. **Marilena Dasenaki:** Writing – review & editing, Methodology, Data curation. **Nikolaos S. Thomaidis:** Writing – review & editing, Resources, Funding acquisition. **Athanasios S. Stasinakis:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180253.

Data availability

Data will be made available on request.

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