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Source investigation of PFAS in home-produced eggs

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Colophon

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Synopsis

Source investigation of PFAS in home-produced eggs

In early 2025, RIVM concluded that high levels of PFAS, higher than in commercial eggs, could be present in home-produced eggs from all parts of the Netherlands. Home-produced eggs are eggs laid by hens that people keep as a hobby, for example in their own back garden or allotment or in a field, therapeutic farm or petting zoo. RIVM has now investigated how PFAS end up in these eggs.

It transpires that the main source of many of the PFAS found in home-produced eggs is earthworms, which chickens consume as part of their natural behaviour. RIVM reached this conclusion because it measured the highest levels of PFAS in home-produced eggs laid by hens that spend time outdoors. The eggs laid by hens kept indoors contained much lower PFAS levels. In addition, RIVM found relatively high levels of PFAS in earthworms. These substances end up in earthworms through the soil. Lastly, the PFAS types found in earthworms matched those found in home-produced eggs.

RIVM is not yet able to state with certainty to what degree other soil fauna (such as spiders, beetles and snails) form a source of PFAS in eggs. This will require further measurements in these animals.

PFAS may also end up in home-produced eggs through other sources in the hens' living environment. RIVM occasionally encountered these substances in water, soil and soil cover such as straw and sawdust, and in other materials, like the wood used to construct the chicken coop and furniture. However, the quantities found were much lower than those found in earthworms and therefore offered no adequate explanation for the high PFAS levels in home-produced eggs.

RIVM has also investigated whether PFAS levels in eggs vary throughout the year. This turns out to be the case, but it is not related to the seasons. Many hens lay fewer or no eggs in winter. Consequently, the expectation was that higher levels of PFAS would be present in the first eggs laid in the subsequent period, but no such effect was found. The causes of the variety in PFAS levels require further study.

Keywords: PFAS, home-produced eggs, earthworms, chickens, measurements, insects, materials, living environment

Publiekssamenvatting

Onderzoek naar de oorzaak van PFAS in particuliere eieren

Begin 2025 concludeerde het RIVM dat er in heel Nederland veel PFAS in particuliere eieren kunnen zitten, meer dan in commerciële eieren. Particuliere eieren komen van kippen die als hobby worden gehouden. Bijvoorbeeld in tuinen, moestuinen, op dierenweitjes en op zorg- en kinderboerderijen. Het RIVM heeft nu onderzocht waardoor de PFAS in deze eieren komen.

Het blijkt dat kippen vooral via wormen in de bodem veel PFAS binnenkrijgen. Kippen pikken de wormen er van nature uit. Het RIVM concludeert dit omdat de meeste PFAS is gemeten in particuliere eieren van kippen die buiten lopen. Er zat veel minder PFAS in eieren van kippen die binnen zitten. Ook vond het RIVM relatief veel PFAS in wormen. De stoffen komen vanuit de bodem in de diertjes. Verder blijken in de wormen dezelfde soorten PFAS te zitten als in de particuliere eieren.

Het RIVM kan nog niet met zekerheid zeggen in welke mate andere kleine bodemdieren, zoals spinnen, kevertjes en slakken, voor de PFAS in eieren zorgen. Daarvoor zijn er meer metingen in deze dieren nodig.

PFAS kunnen ook nog via andere bronnen in de omgeving van de kippen in particuliere eieren terechtkomen. Het RIVM vond de stoffen soms in water, grond en bodembedekkingen als stro en zaagsel, en in ander materiaal, zoals het hout van het kippenhok en meubels. Deze hoeveelheden zijn wel veel lager dan in wormen en kunnen daarom niet de grote hoeveelheden PFAS in particuliere eieren verklaren.

Verder onderzocht het RIVM of de hoeveelheid PFAS in eieren door het jaar heen verschilde. Dat blijkt zo te zijn, maar heeft niet met de seizoenen te maken. Veel kippen leggen in de winter geen of minder eieren. De verwachting was dat PFAS hierdoor meer zouden ophopen in de eerste eieren die ze daarna leggen, dat effect was niet te zien. Meer onderzoek naar de oorzaak van de variatie is nodig.

Kernwoorden: PFAS, particuliere eieren, wormen, kippen, metingen, insecten, materialen, leefomgeving

Contents

1 Introduction

In 2025, the National Institute for Public Health and the Environment (RIVM) assessed the risk of exposure to per- and polyfluoroalkyl substances (PFAS; see Box 1) through the consumption of home-produced eggs from sixty locations in the Netherlands (Nederlof et al., 2025). This study reported PFAS concentrations in the eggs at such high levels that the consumption of eggs from 31 out of 60 locations would result in an exceedance of the health-based guidance value of PFAS, even at the consumption of one egg per week. Therefore, on the basis of the report, it is advised not to consume home-produced eggs in the Netherlands (Nederlof et al., 2025).

Box 1: *PFAS*

Per- and polyfluoroalkyl substances (PFAS) form a large group of synthetic chemicals that have been widely used in industrial applications and consumer products. Known for their unique chemical properties, including resistance to heat, water, and oil, PFAS have been utilised in a variety of products, such as non-stick cookware, water-repellent clothing, stain-resistant fabrics, and firefighting foams. Despite their usefulness, PFAS have raised significant environmental and public health concerns, due to their persistence in the environment and their potential to bioaccumulate in living organisms. Exposure to PFAS can occur through various sources, food and drinking water being the most important sources of exposure in the general population.

The origin of PFAS in home-produced eggs is currently uncertain. In 2024, RIVM assessed the risk of exposure to per- and polyfluoroalkyl substances through the consumption of home-produced eggs from 31 locations, of which 28 were situated in the vicinity of Chemours (Boon et al., 2024). Chemours is a chemical plant in Dordrecht (located in the southwest of the Netherlands) that has emitted perfluorooctanoic acid (PFOA) until 2012 and GenX since then. Also, high PFAS concentrations were detected in these eggs. PFOS was the most found PFAS in these eggs, which has, to our knowledge, not been emitted by Chemours. In the same year, the Office of Risk Assessment and Research (BuRO) of the Netherlands Food and Consumer Product Safety Authority (NVWA) published a risk assessment of PFAS in home-produced eggs in the Netherlands (BuRO, 2024). In this study, no connection could be made with any known source of contamination, such as proximity of industry or other known sources of PFAS contamination.

At the same locations around Chemours as in Boon et al. (2024), potential sources of PFAS in home-produced eggs were investigated (Arcadis, 2024). Types of chicken feed, water, soil, bedding, mealworms, vitamins, medications, and earthworms were investigated. It was concluded that earthworms may be a source of PFAS in home-produced eggs, as these worms could contain high concentrations of PFAS. However, it was not clear whether earthworms are the only source of exposure for chickens (Arcadis, 2024).

Other studies have linked high PFAS concentrations in home-produced eggs to hens consuming kitchen leftovers (Lasters et al., 2022) or fishmeal (Granby et al., 2024), or to exposure to cardboard and dried pulp bedding (Fernandes et al., 2023), and to soil (D’Hollander et al., 2011) and water (D’Hollander et al., 2011). These studies only looked at PFAS, such as PFOS and PFOA, and not at precursors of these PFAS. A PFAS precursor is a PFAS that is can transform into a more stable and persistent PFAS through biological or physical processes in the environment or in organisms. If a hen is exposed to, for example, PFOS precursors, these might end up as PFOS in eggs, and therefore, precursors might be a source of PFAS in eggs.

The Dutch Ministry of Health, Welfare and Sport requested RIVM to investigate the source or sources of PFAS contamination in home-produced eggs, and determine whether there are ways to reduce these PFAS concentrations. In this report, we examine how PFAS contamination in home-produced eggs is related to how hens were kept, using both a source investigation questionnaire at sixty locations and measurements of PFAS and PFAS precursors in potential sources (soil, water, bedding, and earthworms) at ten follow-up locations. Furthermore, we looked into possible seasonal effects of PFAS concentrations in home-produced eggs. Since hens lay fewer eggs during moulting, and stop laying eggs in winter if they are not provided with additional light, we hypothesised that there could be seasonal variation of PFAS concentrations in eggs.

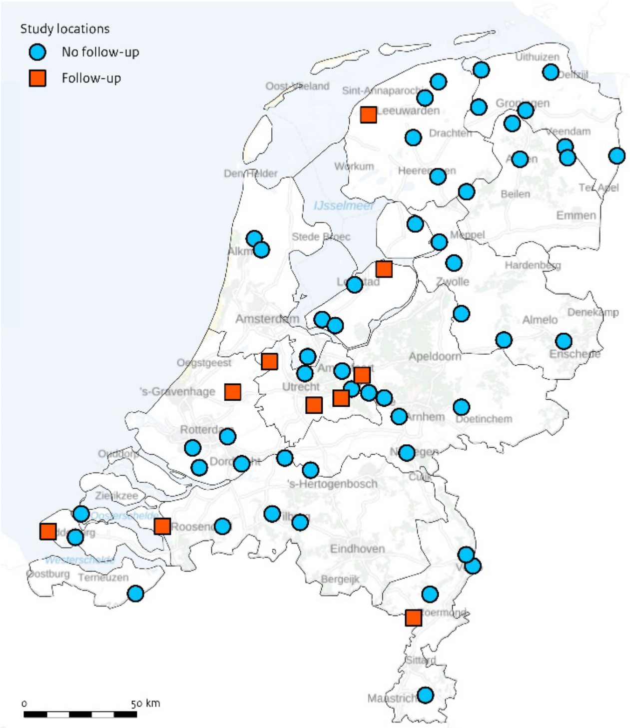
2 Selection, collection and analysis of material and data

2.1 Participant selection for collection of data and materials

As described in Nederlof et al. (2025), sixty chicken owners who keep chickens as a hobby and live in the Netherlands, were recruited via RIVM social media channels during the summer of 2024. Only participants who owned fewer than ten chickens were selected. These participants had filled out an online recruitment questionnaire on the number of hens, chicken housing, and whether their eggs had been tested for PFAS before. Using the online recruitment questionnaire, informed consent was obtained from each participant, including permission for completing a second source investigation questionnaire on how the hens were kept, for sampling of potential source material for investigation of PFAS contamination, and for analysing seasonality in PFAS concentrations in home-produced eggs. These sixty chicken owners were asked to submit five to ten eggs produced by their hens in September 2024, for analysis of PFAS. Nederlof et al. (2025) reported on the PFAS concentrations in that first series of home-produced eggs. Along with the collection of the first series of eggs, participants were asked to complete the online source investigation questionnaire to enable investigation of associations between the PFAS concentration in eggs and how the hens were kept.

Out of the sixty locations, ten follow-up locations were selected for the sampling of potential source material for investigation of PFAS contamination and for analysis of seasonality in PFAS contamination of home-produced eggs (Figure 1). Sampling of source material took place in autumn, which means that some hens were moulting and not laying eggs. Therefore, locations were selected from chicken owners who reported that their hens still laid eggs. To obtain a variance of PFAS concentrations in eggs, which is required to enable detection of associations with possible sources, the ten follow-up locations were selected on their spread across the country, with low, medium and high concentrations of PFAS in the eggs collected in September 2024.

Figure 1 Map of the sampling locations in the Netherlands.



The blue dots and the red squares depict the sixty locations where the first series of home-produced eggs were collected (equal to Nederlof et al. (2025)), as well as the recruitment questionnaire and source investigation questionnaire. The red squares represent the ten follow-up locations where additional sampling was performed for source investigation and seasonality analyses of PFAS contamination in home-produced eggs.

2.2 Collection of home-produced eggs and potential source material from ten follow-up locations

The sampling periods are shown in Table 1. Participants were asked to send five to ten eggs per sampling period to Wageningen Food Safety Research (WFSR) at Wageningen University & Research (WUR). The WFSR laboratory analysed the eggs for PFAS (see section 2.3). Participants sent the home-produced eggs to the laboratory by post in provided cardboard shipping boxes (25 x 25 x 11 cm) containing foam rubber with holes for eggs (Nederlof et al., 2025)

Table 1 Sampling periods in this study.

Sampling period		Sample type	Number of locations
Nr	Months		
1	September 2024	Eggs	60
		Source material	3[1]
2	October–November 2024	Eggs	8
		Source material[2]	7
3	December 2024–April 2025	Eggs	10
4	May–June 2025	Eggs	10

[1]At three locations, sampling period 1 coincided with the collection of source material and no extra eggs were collected at two of these locations during sampling period 2.
[2]Eggs and source material were collected simultaneously.

In September 2024 (sampling period 1), one series of home-produced eggs was collected at sixty locations. At the ten selected follow-up locations, potential source material and further eggs were collected by the research and consultancy firm Arcadis, as described below in this section. At seven out of ten locations, source material was collected simultaneously with the second series of eggs, in October and November 2024 (sampling period 2), at the end of the egg-laying season. At three out of ten locations, source material and eggs were collected in September 2024, during sampling period 1. No eggs were collected during sampling period 2 at two of these three locations. Between December 2024 and April 2025 (sampling period 3), participants sent in the first eggs of the laying season. Most 'first eggs of the laying season' were collected in January 2025, and at two locations, the eggs were collected in March and April 2025. During this sampling period, additional information was collected via email on whether the hens had stopped laying eggs between sampling periods 2 and 3. In May and June 2025 (sampling period 4), which is in the middle of the egg-laying season, additional eggs were collected from all ten follow-up locations.

Arcadis visited the ten follow-up locations to collect a wide range of source materials from the surroundings of the hens. Sampling strategies were tailored to each location, as they varied in chicken housing, ground cover and feed. Site-specific sampling plans were designed using information from the source investigation questionnaire, from observations of local conditions, and from conversations with the chicken owners during the site visit.

The following potential source materials were sampled for the analysis of PFAS: water (i.e. tap water, surface water from pond, rain water, and/or groundwater), soil and earthworms from both inside and outside the coop where chickens forage, insects, bedding material, and miscellaneous samples, such as shavings from impregnated and unimpregnated wood from, for example, the coop, posts or outdoor furniture, grit of shells, lime fertiliser, twigs, and anti-lice medication.

Soil sampling was conducted according to Dutch standard BRL2001 and the Dutch PFAS sampling procedure.[1] The BRL2001 standard contains procedures for the collection, handling, and documentation of soil samples. Samples from the topsoil included the layer accessible to hens (0–5 cm below ground level), the rooting zone (5–20 cm below ground level), and the layer beneath the rooting zone (>20 cm below ground level). Earthworms were sampled right next to the soil drillings. After drilling for soil, a hole was made with a volume of 30 x 30 x 20 cm from which the earthworms were collected.

Each sample of potential source material was assigned a unique code, and photographs were taken to document site conditions and sample locations. Samples were collected in plastic jars or PFAS-free HDPE freezer bags. All samples were sent to WFSR, where they were stored until analysis. Additional material was sampled on site, including chicken feed, other feed, such as kitchen waste, packaging material of the

[1] https://www.sikb.nl/doc/BRL2000/Protocol_2001_v6_0_20180201.pdf

chicken feed, tarp on top of chicken coop, twigs, and feathers. These samples were stored for potential later analyses.

2.3 Analysis of PFAS in eggs

Appendix 1 lists the names and abbreviations of PFAS compounds analysed in this study. The analysis of PFAS was performed as described in Boon et al. (2024) and Nederlof et al. (2025). WFSR analysed the composite samples for 18 PFAS (see Appendix 1). These 18 PFAS were chosen because they are included in the analytical method applied by WFSR. The eggs were analysed raw. Prior to the analysis, eggshells were removed, and the eggs (yolk and egg white) were pooled per location and homogenised.

The 18 PFAS were analysed according to WFSR's internal Standard Operating Procedure (SOP) A1114. One gram per sample was weighed. Following extraction with acetonitrile and purification via Solid Phase Extraction, the sample extracts were analysed using liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS).[2] The analytical LC column was a Phenomenex Luna Omega 1.6 µm PS C18 100 Å (100 x 2.1 mm) and a Phenomenex Gemini 3 µm C18 110 Å (50 x 3 mm) isolator column. The solvents used to pass the sample extracts through the column were mobile phases A and B (A: 20 mM ammonium acetate in water; B: 100 percent acetonitrile; flow rate 0.65 ml/min). The MS operated in multiple reaction monitoring, where each PFAS is monitored on the basis of two ion transitions (with the exception of PFBA and PFPeA, because only one ion transition is available for these PFAS). The amount of each PFAS was quantified using calibration curves, prepared in the presence of the matrix.[3] If possible, the calibration curves were made according to isotope-labelled internal standards for the linear isomers of PFAS. Since most PFAS in food are present as linear isomers, the concentrations of each isomer are reported. However, PFHxS, PFOA, and PFOS can also be present in food as branched isomers. No branched isomers were observed for PFHxS in the samples. Branched isomers of PFOA were present, but the concentrations were too low to distinguish them from the background signal. For PFOS, branched isomers were quantified using the calibration curves for the linear isomers as described in the guidance document on the analysis of PFAS.[4] While this may result in a small inaccuracy in the reported concentrations of PFOS (linear and branched), it is expected to be minimal.

2.4 Analysis of PFAS in source material

For the analysis of the source material, the samples were divided into four categories: earthworms and insects, soil, water, and bedding and miscellaneous. All samples were analysed on 39 (not necessarily the same) PFAS (see Appendix 1). The selected 39 PFAS comprised 18 PFAS that were also analysed in home-produced eggs by WFSR (see section 2.3). The remaining 22 PFAS were precursors of PFOS, longer-

[2] LC-MS/MS, LC: Shimazdu ExionLC AD; MS: Sciex 7500.
[3] The matrix includes all other substances in a sample besides the substance to be analysed.
[4] EURL for halogenated POPs in Feed and Food (2024): Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed, version 2.0 from 10 September 2024. https://eurl-pops.eu/working-groups#_pfas

chain sulfonic acids (PFUnDS, PFDoDS and PFTrDS), fluorotelomer sulfonates (FTSs), and other PFAS (e.g. PFECHS, 8Cl-PFOS).

For earthworms, soil, and water, multiple samples were measured per location, if available (see Appendices 2–4).

The samples were ground to reach homogeneity. In some cases, it was not possible to obtain a homogeneous sample. This was particularly the case for samples for which insufficient source material was provided, as well as for those in the bedding and miscellaneous category.
For the sample categories earthworms and insects, soil, and bedding and miscellaneous, one gram per sample was weighed. For the sample category water 200 grams per sample was weighed. In cases where insufficient sample material was provided, the sample weight was adjusted accordingly, resulting in an increased limit of quantification (LOQ) for these samples. For insects, such as spiders, woodlice, and small garden slugs, the sample volume was too small. Therefore, insects from the various locations were pooled into one sample.

To every series, a representative sample was spiked at at least 3 levels to monitor quality of the series, to establish the LOQ and to check for matrix effects (earthworms/ insect/ soil/ bedding and miscellaneous: 50, 100, 250, and 400 pg/g, water: 0.1, 0.5, and 1 ng/L). The samples were extracted with acetonitrile (for the earthworms and insects and soil categories) or methanol (for bedding and miscellaneous). Water samples were analysed without extraction. Prior to the clean-up, the extracts and water samples were acidified using formic acid. The clean-up was performed with solid phase extraction (SPE) (Strata-X-AW Phenomenex). The extracts were analysed using liquid chromatography (LC) coupled with a tandem mass spectrometer (MS/MS) (LC: Shimadzu ExionLC AD; MS: Sciex QTRAP 7500).

Chromatographic separation was obtained by using a Phenomenex Luna PFP (2) analytical column (100 Å, 150 × 2 mm inner diameter, 3 µm) at a column temperature of 40 °C. A Phenomenex Gemini C18 (110 Å, 50 × 3 mm inner diameter, 3 µm) isolator column was used to remove PFAS contamination from the system and mobile phases. For mobile phase A, 20 mM ammonium acetate in water was applied, for mobile phase B, acetonitrile/methanol mixture (1:1) was applied. The flow rate was maintained at 0.4 mL/min, and the injection volume was 20 µl.

The MS operated in multiple reaction monitoring, where each PFAS is monitored on the basis of two ion transitions (with the exception of PFBA and PFPeA, because only one ion transition is available for these PFAS). The amount of each PFAS was quantified using the calibration curve of the respective PFAS compounds in solvent. If possible, the calibration curves were made according to isotope-labelled internal standards for the linear isomers of PFAS. If present, branched isomers were quantified using the calibration curves for the linear isomers as described in the guidance document on the analysis of PFAS. While this may result in an inaccuracy in the reported concentrations, it is expected to be minimal.

In soil samples, the dry matter content was determined by means of ISO 11465:2025. Five grams of each sample were weighed. Samples were dried to constant mass in an oven at (105 ± 5) °C. The difference in mass before and after the drying process was used to determine the dry matter content. The organic matter content was determined by loss on ignition (based on NEN-5754 and NEN-EN-15935). The samples were pretreated by drying at 40 °C and sieving (2 mm sieve). The samples were weighed before ignition, then ignited at 550 °C and weighed again. The percentage of organic matter was calculated by dividing the residual weight after ignition by the original weight.

2.5 Data analysis of PFAS concentrations in eggs and source material

Concentration data was provided as numerical values, below LOQ, not detected (n.d.) or as a '+'.

The LOQ is the lowest concentration of a PFAS that can be quantified with established precision and at which the identity of the PFAS can be confirmed. These limits varied per PFAS, because these substances do not have the same chemical properties, which affects their behaviour in the LC-MS/MS. Long-chain PFAS (such as PFOA and PFOS) tend to adhere to the LC column more than short-chain PFAS (such as PFBA and PFBS). Furthermore, due to analytical reasons, the LOQ for a PFAS may not always be the same within a run or between different matrices. Therefore, the LOQ for a PFAS may differ between series and samples (Appendices 2–4, 7, and 8). In the current analyses, the LOQs in eggs ranged from 0.01 to 0.40 nanogram (ng) per gram, in earthworms/insects from 0.025 to 0.4 ng per gram, in soil from 0.05 to 0.25 ng per gram, in water from 0.0001 to 0.005 ng per mL, and in soil cover material from 0.00010 to 0.25 ng per gram. Table 2 shows how concentrations were reported and how they were allocated in the calculation of the sum concentration of PFAS. To calculate sum concentrations, reported concentrations ('c') were added together. This allocation is equal to the so-called 'lower bound' scenario.

Table 2 PFAS concentrations allocated to the reported concentrations in the graphs and summation calculations.

	Reported concentration	**Allocated concentration in summation calculations**
Limit of Quantification: concentration in the sample is too low to be reliably measured and reported by the analytical method used. The compound may be present, but the amount is so small that the results cannot be quantified with the necessary accuracy and precision. The concentration of the respective PFAS is between 0 ng per gram and the LOQ.	< LOQ	0

	Reported concentration	**Allocated concentration in summation calculations**
Numerical concentration: the PFAS was detected and the concentration could be accurately quantified; the concentration of the respective PFAS is equal to 'c'.	c	c
Not determined: the PFAS could not be determined	n.d.	Not included
+*:* the PFAS was detected above LOQ, but due to an unstable calibration curve, the concentration could not be quantified	+	Not included

c: numerical concentration; LOQ: limit of quantification; n.d.: not determined; PFAS: per- and polyfluoroalkyl substances.

PFAS concentrations measured in soil are presented both in ng per gram dry matter and normalised for percentage organic carbon. PFAS concentrations were normalised for percentage organic carbon by the formula[5]:
$\text{Concentration}_{\text{corrected}} = \text{Concentration}_{\text{measured}}$ * (10% organic matter standard soil/% organic matter measured).
Normalisation to organic carbon is used to reduce variability in data on how chemicals bind to soil. This is based on the idea that most chemicals stick to the organic carbon in soil, especially through hydrophobic (water-repelling) interactions. Since it is not clear whether this approach is appropriate to ionogenic substances like PFAS, in the main text, PFAS concentrations in soil are shown in ng per gram dry matter. Concentrations normalised for organic carbon are shown in Appendix 3.

Summed and single PFAS concentrations were compared between source material and eggs from the same locations. When locations had more than one sample within one category (e.g. two earthworm samples on one location, or multiple soil samples), the mean concentration was calculated. No mean was calculated for bedding and miscellaneous material and water, since the source material was too diverse (for example, sawdust and scraping of chicken coop).

Associations between concentrations of PFAS in eggs and in source material, and concentrations of PFAS in eggs across the four sampling periods are visualised in bar charts, scatterplots and boxplots. PFAS concentrations do not follow a normal distribution, so analyses of PFAS concentrations were performed using non-parametric methods. Spearman's non-parametric correlations were calculated for statistical significance in scatterplots, and Kruskal Wallis tests were applied to test for statistical differences between PFAS concentrations in boxplots. All data wrangling, analyses and visualisations were done in either Excel or

[5] https://rvs.rivm.nl/sites/default/files/2018-05/Toelichting%20mei%202011%20%281%29.pdf

R (packages xlsx, writexl, janitor, tidyr, dplyr, ggplot2, palletteer, tidyverse, Hmisc, fastDummies, glmnet).

2.6 Preparation and analysis of questionnaire data for associations with PFAS concentrations

Using the online recruitment questionnaire, information was collected on the addresses of the participants, the amount of hens they kept, the types of housing (free-range or housed within a coop), and whether eggs had been tested for PFAS before. With the second online self-administered source investigation questionnaire, information on how the hens were kept was collected through sixty questions, covering the following six topics: specifications on the hens, egg-laying frequency, feed and water intake, the space and soil where hens forage, the coop, and medication use. The questions in the source investigation questionnaire are provided in Appendix 5.

Preceding the data analysis, the source investigation questionnaire data was cleaned, meaning that free written texts, and some answering categories were pooled into combined answering categories. Missing answers, which occurred rarely, were assigned to ‘missing’ categories, unless the information on that question could easily be deduced from other questionnaire answers or the free written text. Numerical variables were categorised as tertiles or quantiles, according to the observed data distribution in scatterplots.

Associations between various PFAS concentrations in potential sources and in home-produced eggs from the first sample period were assessed using multivariable linear LASSO regression (Appendix 6). Using this statistical method we determined which variables entered into the model were the best predictors of PFAS concentration in eggs. To keep the statistical model simple and prevent overfitting, the LASSO model applies a penalty to ignore less important variables. All variables from the source investigation questionnaire were entered into the LASSO regression model as independent variables and the summed PFAS concentration in eggs as dependent variable. The summed concentrations of PFAS in eggs were log-transformed (log(value+1)) to include the location with a summed PFAS concentration of 0 in the analysis and to attain a near-normal distribution of this variable.

3 Results

3.1 Associations between PFAS concentrations in eggs and in potential source material

Appendices 2 to 4, 7, and 8 provide the PFAS concentrations in eggs and samples of the different source categories.
This includes:

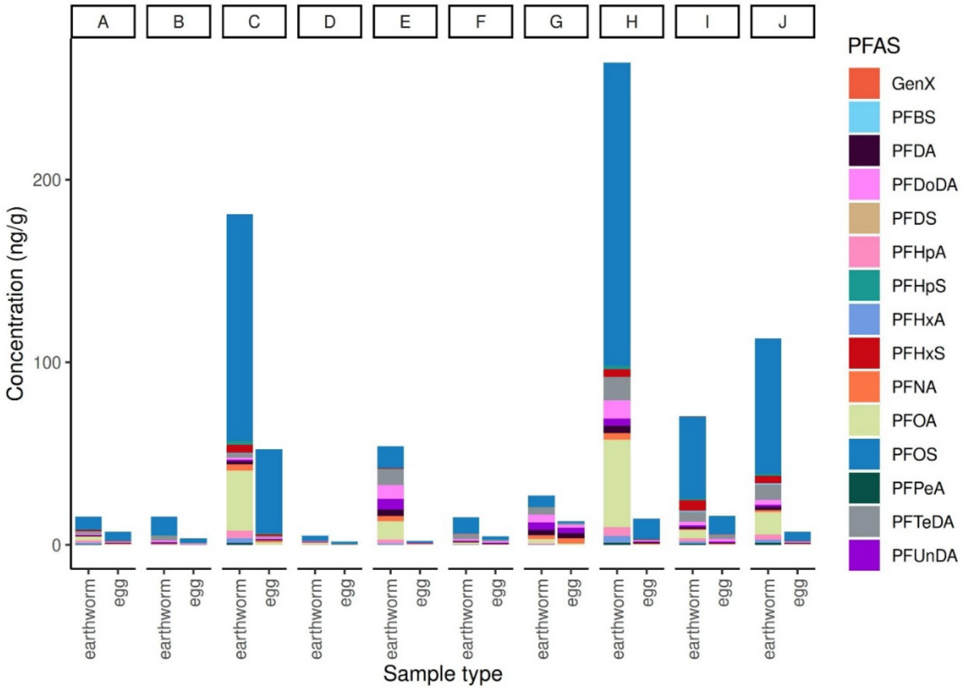
- PFAS concentrations in home-produced eggs at sixty locations, as previously reported by Nederlof et al. (2025) (Appendix 7).
- PFAS concentrations in home-produced eggs at ten follow-up locations obtained during sampling periods 3 and 4 and eight locations during sampling period 2 (Appendix 7); and
- PFAS and precursor concentrations at ten follow-up locations in the four potential source material categories (see section 2.4):
  1) earthworms and insects (Appendix 2);
  2) soil (Appendix 3);
  3) water (Appendix 4);
  4) bedding and miscellaneous (Appendix 8).

Appendix 9 lists the main contributing PFAS compounds per location for eggs, earthworms, soil and water, expressed as percentage contribution of individual PFAS compounds to the summed concentrations of PFAS per egg/source.

Concentrations of 16 PFAS in eggs and source material samples were compared (Appendix 1): PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS, PFDS, GenX and ADONA. PFTrDA was not included in the comparison with earthworms, since this PFAS was reported as '+' in earthworms.
As mentioned in section 2.4, source material samples were analysed for an additional 21 (earthworms and soil) or 22 (water and bedding and miscellaneous) PFAS; longer-chain sulfonic acids (PFUnDS, PFDoDS and PFTrDS), fluorotelomer sulfonates (FTSs), and other PFAS (e.g. PFECHS, 8Cl-PFOS) (Appendix 1). However, these PFAS-precursors were mostly reported as either not detected or below LOQ. The concentrations for these PFAS are shown in Appendices 2–4 and 8 and will not be discussed further.

PFAS concentrations in earthworms, and comparison with PFAS concentrations in eggs
High concentrations of PFAS were reported in the pooled earthworm samples at each of the ten follow-up locations, with summed concentrations ranging from 5.1 to 264 ng per gram (Figure 2). In the eggs collected in the same sampling period, summed concentrations of PFAS ranged from 1.7 to 52 ng per gram. The summed PFAS concentrations in earthworms were a factor of 2.1 to 24 higher than in eggs.

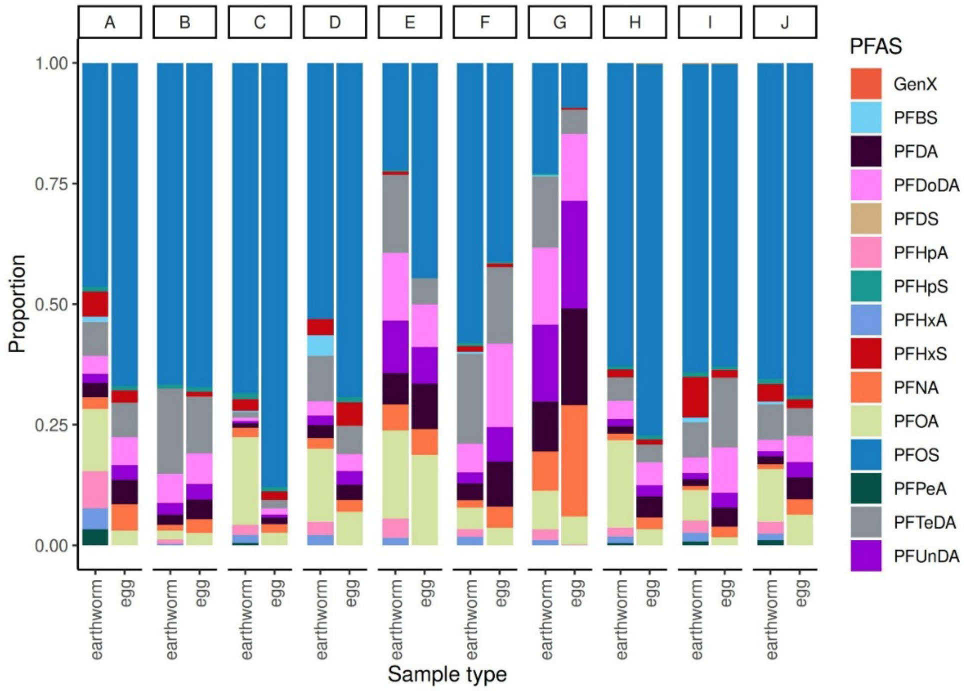
Figure 2 Concentrations of PFAS in nanogram per gram in earthworms and eggs at the ten follow-up locations.



PFAS are depicted when analysed and reported in earthworms as well as in eggs at a concentration above the limit of quantification. On the y-axis, PFAS concentration in nanogram (ng) per gram is displayed, while the x-axis displays the sample type. Colours annotate the various PFAS, and the facet letters on top (A–J) represent the locations. See Appendices 2 and 7 for the PFAS concentrations in earthworms and eggs, respectively. PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).

Figure 3 and Appendix 9 present the detected PFAS as a fraction of the summed concentrations of PFAS per location. At each location, the proportion of PFAS compounds in eggs closely resembled that in earthworms. This was also the case for location G, even though the PFAS proportions at this location displayed an irregular pattern with mainly a smaller fraction of PFOS (9% in eggs and 23% in worms) than at the other 9 locations. At these 9 locations, PFOS was the main contributor to the summed PFAS concentrations in eggs, ranging from 41% to 88% of the summed total PFAS concentration. Correspondingly, PFOS was also the main contributor in earthworms, ranging from 22% to 68%. PFOA was the second main contributor in eggs in four out of ten locations ranging from 3% to 19% of the summed total PFAS concentration. In earthworms, PFOA was the second-largestcontributor in 6 out of 10 locations ranging from 11% to 18%. The next most detected PFAS in eggs and earthworms at all locations was PFTeDA at 2% to 16% in eggs, and 1% to 19% in earthworms (regardless of contributor rank).

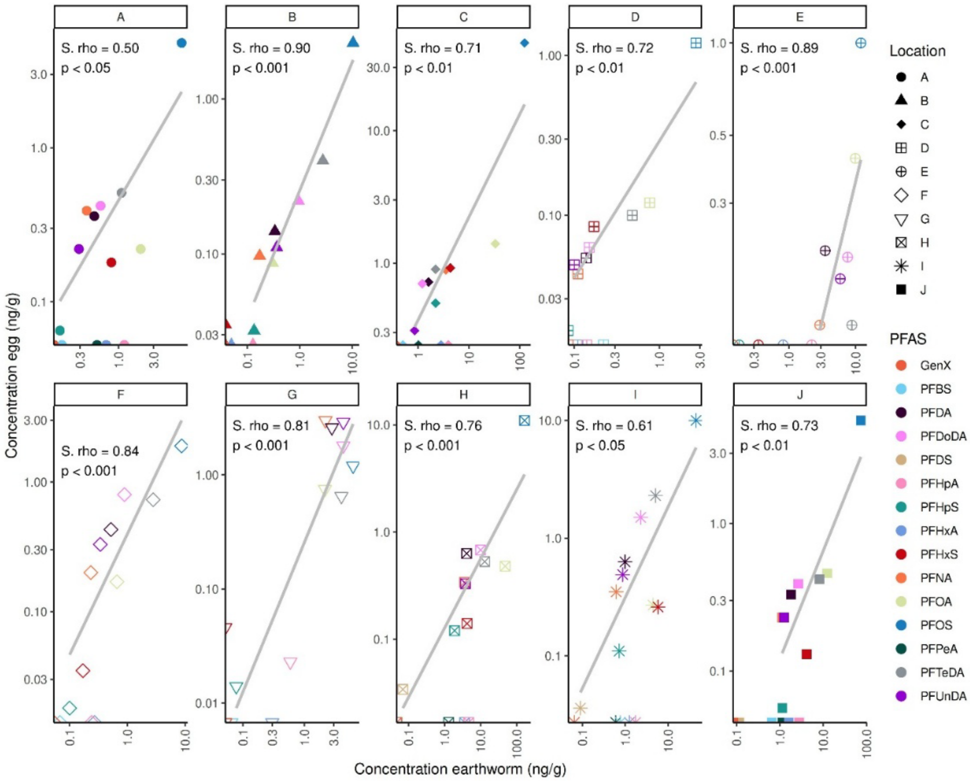
Figure 3 Proportion of PFAS in earthworms and eggs at the ten follow-up locations.



PFAS are depicted when analysed and reported in earthworms as well as in eggs at a concentration above the limit of quantification. On the y-axis the proportion is displayed, while the x axis presents the sample type. Colours annotate the PFAS, and the facet letters on top (A–J) represent the locations. See Appendix 9 for the top 3 PFAS contributions in eggs and earthworms.
PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).

As depicted in Figure 4, scatterplots per location of the concentrations in earthworms and eggs demonstrate that high concentrations of PFAS in earthworms coincided with high concentrations of these PFAS in eggs within locations, and lower concentrations of PFAS in earthworms with lower concentrations in eggs. Spearman's non-parametric correlation statistics showed strong and statistically significant correlations between PFAS concentrations in earthworms and eggs at all locations (Spearman's rho coefficients ranged from $\rho = 0.50$ to $\rho = 0.90$; Figure 4). The observed correlations are in agreement with our observation that the proportion of PFAS compounds in eggs closely resembled that in earthworms (Figure 3).

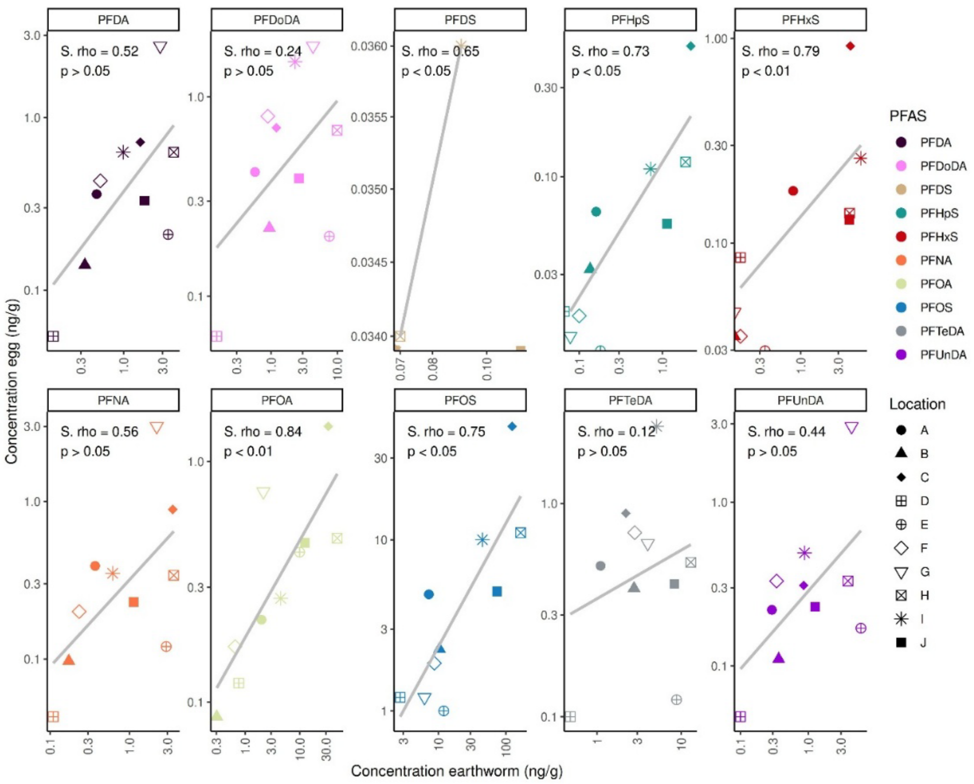
Figure 4 Scatterplots with Spearman's correlations showing the relationship between PFAS concentrations in earthworms and eggs at the ten follow-up locations.



Each panel represents a location (as indicated by the facet labels A–J in the box above the panel and the shape of the data point), with data points coloured by PFAS. Both axes of PFAS concentration (ng/g) are displayed on a logarithmic scale. A regression line (grey) is fitted to the data in each panel. The correlation coefficient between PFAS concentrations in earthworms and eggs is reported for each location as Spearman's rho (ρ, S. rho) with p-value (p) to indicate the statistical significance. All 16 PFAS are included in the correlation test and value 0 was used for concentrations below the limit of quantification (LOQ). The figure only shows PFAS with at least one concentration above LOQ in either earthworms or eggs.
PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).

In Figure 5, we explored correlations of concentrations for each PFAS compound in earthworms and eggs across locations, to investigate which PFAS drive the correlations of PFAS concentrations in eggs and earthworms observed in Figures 3 and 4. As depicted in Figure 5, scatterplots of the PFAS concentrations in earthworms and eggs show the correlations separately for each PFAS. For five PFAS, the correlations were statistically significant: PFOA (ρ= 0.84), PFHxS (ρ= 0.79), PFOS (ρ= 0.75), PFHpS (ρ= 0.73), and PFDS (ρ= 0.65). These correlation analyses suggest that earthworms are a likely source of these five PFAS in home-produced eggs. The lowest (not statistically significant) correlation was seen for PFTeDA (ρ=0.12), even though this PFAS was detected in all eggs and worms and contributed up to 2–16% in eggs and 1–19% in worms. This suggests that PFTeDA may not be transferred efficiently from earthworms to eggs and/or that worms may not be the main source of PFeDA in eggs. For ADONA, GenX, PFBS, PFHxA, PFPeA and PFHpA no meaningful statistics could be obtained due to fewer than two concentrations above LOQ in eggs or earthworms. See also the end of section 3.1 for an analysis of correlations between eggs, worms, soil, and water for PFOS and PFOA.

Figure 5 Correlation between PFAS concentration in earthworms and eggs for ten individual PFAS.



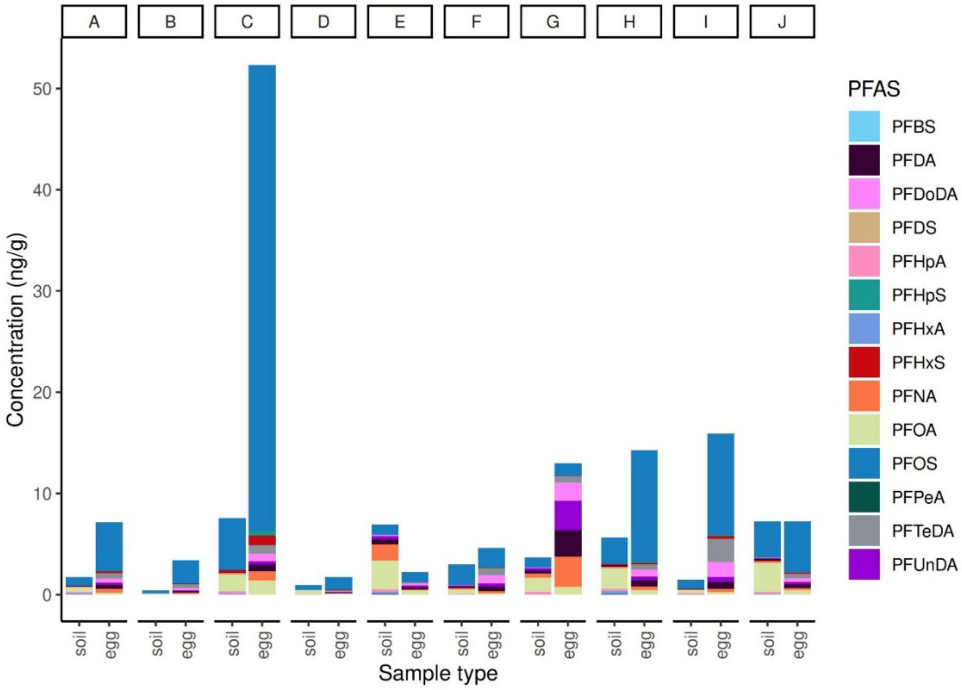
Scatterplots show the relationship between PFAS concentrations in earthworms and eggs for individual PFAS. Each panel represents a specific PFAS (as indicated by the facet labels), with data points coloured by PFAS and shaped according to location. Concentrations are expressed in ng/g and are displayed on a logarithmic scale. A regression line (grey) is fitted to the log-transformed data in each panel. Correlation statistics for each PFAS (as determined in a non-parametric correlation analysis) are shown as text annotations in the upper left corner of each facet. All ten locations are included in the correlation test and the value 0 was used for concentrations below the limit of quantification (LOQ). The figure shows only PFAS with at least one concentration above LOQ in either worms or eggs. S. rho = Spearman’s rho, p = p-value.
PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).

PFAS concentrations in insects, and comparison with PFAS concentrations in eggs

One pooled insect sample was collected as a pilot sample to explore if PFAS could be detected in insects. As shown in Appendix 2, the insect sample contained 23 ng/g PFOS, 21 ng/g PFDoDA, 11 ng/g PFDA, 6.9 ng/g PFTeDA, 5 ng/g PFOA, 4.1 ng/g PFNA, 3.8 ng/g PFUnDa, 0.72 ng/g PFHpA, 0.23 ng/g PFHxA, 0.61 ng/g PFHxS, and 0.36 ng/g PFHpS. These concentrations were relatively high, suggesting that insects may play a role in the intake of PFAS by hens. However, since this is only one pooled sample of mixed insect specimens from several locations, no conclusion can be drawn on the contribution of insects to PFAS in home-produced eggs.

PFAS concentrations in soil, and comparison with PFAS concentrations in eggs
PFAS were reported in the pooled soil samples from all locations at summed concentrations ranging from 0.44 to 7.58 ng per gram (Figure 6 and Appendix 3). Compared to PFAS concentrations in eggs (Figure 6), PFAS concentrations in soil were lower at eight locations, with summed concentrations being a factor 1.5 to 11 lower in soil than in eggs. At locations J and E, summed PFAS concentrations in soil were the same as or 3.1 times higher than in eggs, respectively. Soil concentrations normalised for organic carbon are shown in Appendices 3 and 10.
At locations B and C, sand was collected from the chicken coops. PFAS was only quantified in sand from location C: PFOS at 5.3 ng/g dry matter, PFOA at 0.5 ng/g dry matter, and PFOSA, PFHxS, PFNA, PFDA and PFUnDA between 0.26–0.06 ng/g dry matter (see Appendix 3).

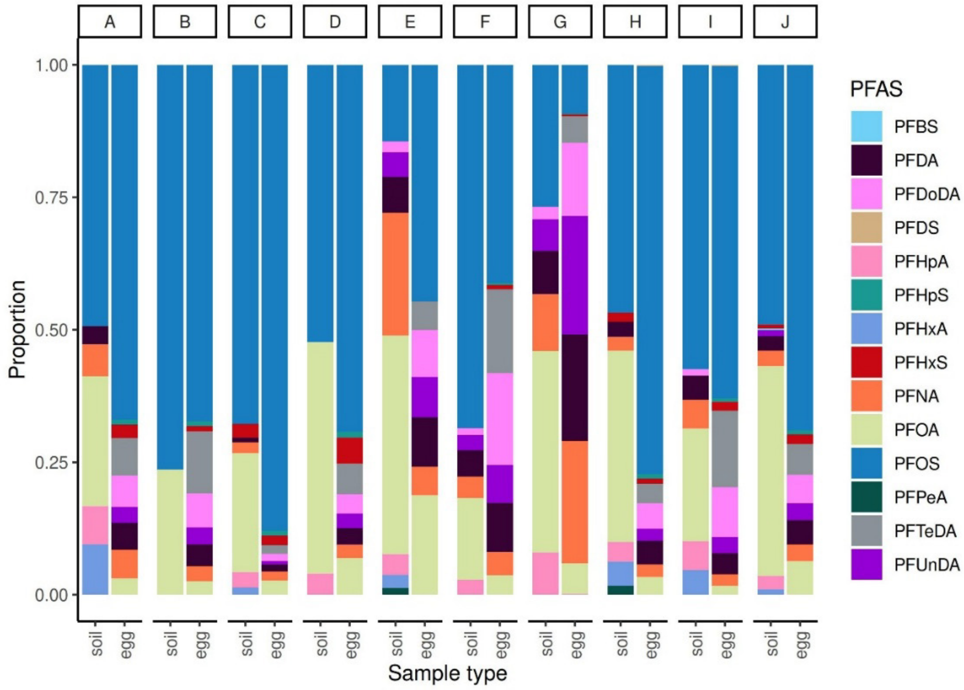
Figure 6 Concentrations of PFAS in nanograms (ng) per gram in soil (mean concentrations of soil of different PFAS; see section 2.4) and eggs at the ten follow-up locations.



PFAS are depicted when analysed and reported in soil as well as in eggs at a concentration above the limit of quantification. On the y-axis, PFAS concentrations in nanogram per gram are displayed (ng per g dry matter for soil), while the x-axis displays the sample type. Colours annotate the PFAS, and the facet letters on top (A–J) represent the locations. See also Appendices 7 and 3 for the PFAS concentrations in eggs and soil, respectively.
PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).

Figure 7 and Appendix 9 present the detected PFAS as a fraction of the summed concentrations per location. At each location, the proportion of PFAS in eggs showed some resemblance to that in soil. Also, in soil, PFOS was the main contributor to the summed PFAS concentrations at eight locations, ranging from 47% to 76%. The next most detected PFAS in soil were PFOA and PFNA (see Appendix 9 for the contribution percentages).
See also the end of section 3.1 for an analysis of correlations between eggs, worms, soil and water for PFOS and PFOA.

Figure 7 Proportion of PFAS compounds in soil and eggs at the ten follow-up locations (see also Appendix 9).

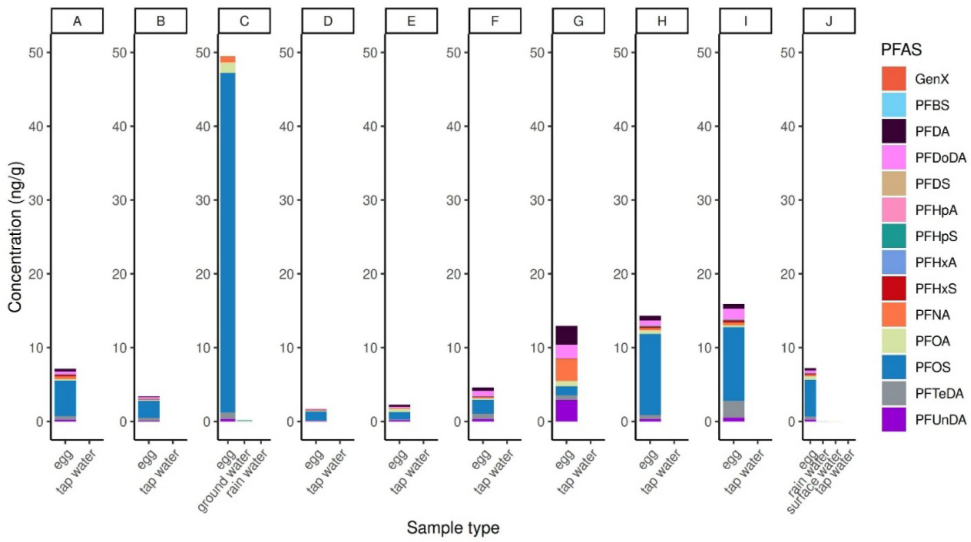


PFAS are depicted when analysed and reported in soil as well as in eggs at a concentration above the limit of quantification. On the y-axis the proportion is displayed, while the x-axis displays the sample type. Colours annotate the PFAS, and the facet letters on top (A–J) represent the locations. See Appendix 9 for the top 3 PFAS contributions in eggs and soil. PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).

PFAS concentrations in water, and comparison with PFAS concentrations in eggs

The data analyses of water samples focused on the sources (tap, rain, surface and ground water) accessible to the hens, as determined from the questionnaire responses. PFAS could be quantified in the water samples at four locations (C, F, G and J), at levels 242 to 10062 times lower than in the eggs at those locations (Figure 8 and Appendix 4). Ground water and surface water contained higher summed concentrations than rain and tap water. At locations F and G, the summed concentrations were less than 0.001 ng per mL and at locations C and J, they amounted to 0.19 and 0.01 ng per mL, respectively.

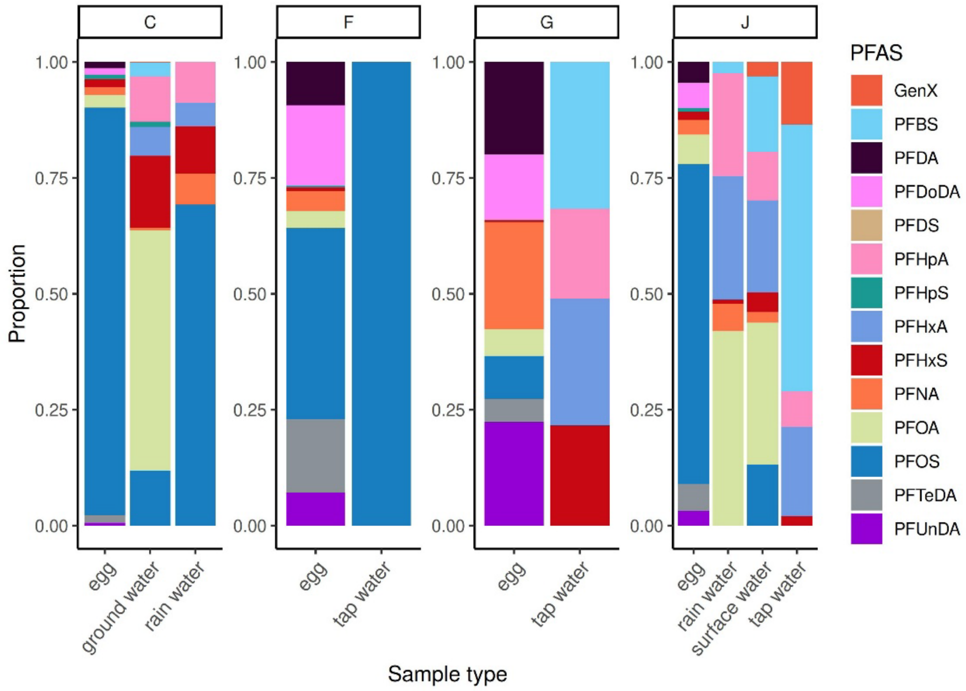
Figure 8 Concentrations of PFAS in nanogram (ng) per gram in water sources accessible to hens, and in eggs at the ten follow-up locations.



PFAS are depicted when analysed and reported in water as well as in eggs at a concentration above the limit of quantification. On the y-axis, PFAS concentrations in nanogram per gram are displayed, while the x-axis displays the sample type. Colours annotate the PFAS, and the facet letters on top (A–J) represent the locations. See also Appendices 7 and 4 for the PFAS concentrations in eggs and soil, respectively.
PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).

Figure 9 and Appendix 9 present the detected PFAS compounds as a fraction of the summed concentrations per location. At the four locations where PFAS was detected in water, the PFAS in eggs showed very little resemblance to the proportions in water.
See also the end of section 3.1 for an analysis of correlations between eggs, worms, soil and water for PFOS and PFOA.

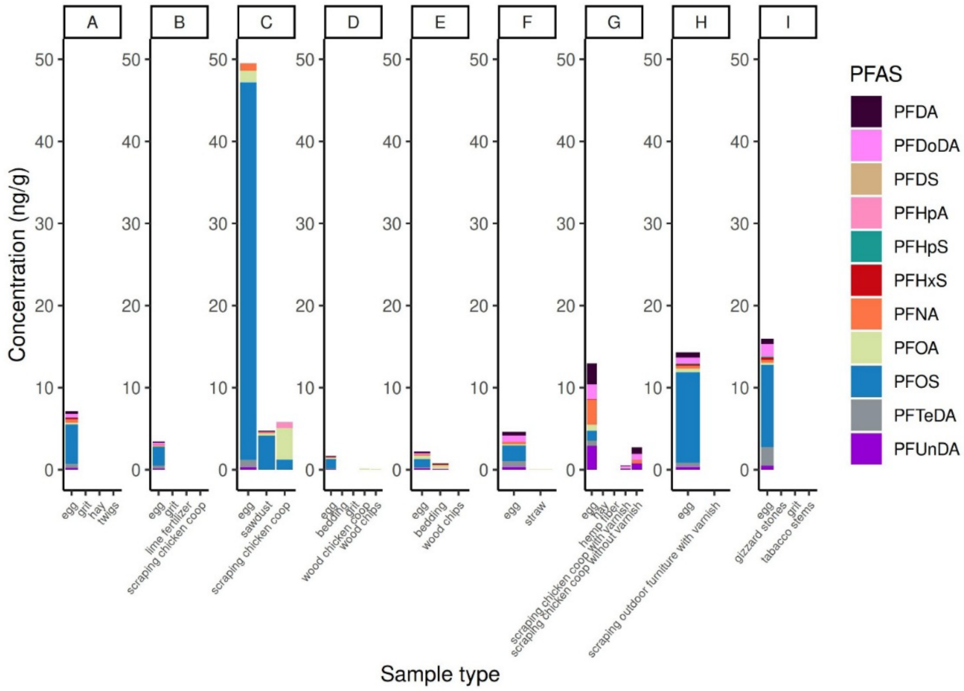
Figure 9 Proportion of PFAS compounds in water and eggs at four follow-up locations where PFAS was detected and reported.



PFAS are depicted when analysed and reported in water as well as in eggs at a concentration above the limit of quantification. On the y-axis, the proportion is displayed, while the x-axis displays the sample type. Colours annotate the PFAS, and the facet letters on top (A–J) represent the locations. See Appendix 9 for the top 3 PFAS contributions in eggs and water.
PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).

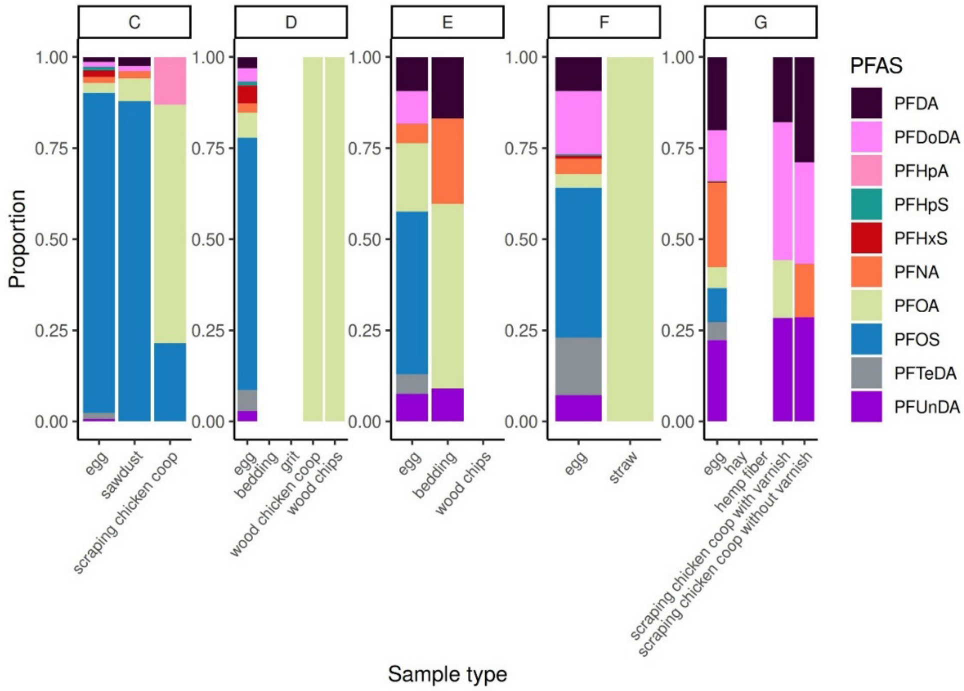
PFAS concentrations in bedding and miscellaneous, and comparison with PFAS concentrations in eggs
Different types of bedding were sampled at nine locations, including wood chips, hay, bedding, straw, and grit. Out of the 17 PFAS analysed in the eggs, few were reported above the LOQ in bedding and miscellaneous at five locations (see Figure 10 and Appendix 8). The highest PFAS concentrations in these materials were found at location C in sawdust and scraping material from the chicken coop. PFOS and PFOA were mainly present in sawdust, (4.2 and 0.30 ng/g, respectively) and PFOA, PFOS and PFHpA were found in the scraping material (3.8, 1.24, and 0.76 ng/g, respectively).
At location G, other PFAS were found in the chicken coop with and without varnish. The sample without varnish contained PFDA (0.79 ng/g), PFUnDA (0.78 ng/g), PFDoDA (0.76 ng/g), PFTrDA (0.54 ng/g), and PFNA (0.4 ng/g), whereas the one with varnish contained PFDoDA (0.2 ng/g), PFTrDA (0.15 ng/g), PFUnDA (0.15 ng/g), PFDA (0.095 ng/g), and PFOA (0.084 ng/g), all at lower concentrations.
Except for locations C and G, the pattern of PFAS present in the bedding and miscellaneous samples showed little resemblance to the PFAS in eggs (Figure 11).

Figure 10 Concentrations of PFAS in nanogram (ng) per gram in bedding and miscellaneous samples accessible to hens, and in eggs at nine follow-up locations.



PFAS are depicted when analysed and reported in bedding and miscellaneous as well as in eggs at a concentration above the limit of quantification. On the y-axis, PFAS concentrations in nanogram per gram are displayed, while the x-axis displays the location and sample type. Colours annotate the PFAS, and the facet letters on top (A–J) represent the locations. See also Appendices 7 and 8 for the PFAS concentrations in eggs and bedding and miscellaneous, respectively.
PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).

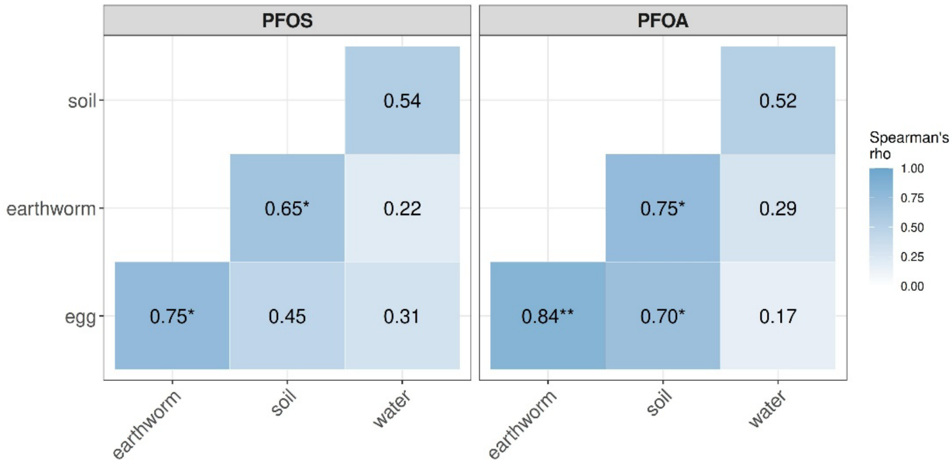
Figure 11 Proportion of PFAS in bedding and miscellaneous and in eggs at five follow-up locations where PFAS was detected and reported.



PFAS are depicted when reported in bedding and miscellaneous as well as in eggs at a concentration above the limit of quantification. On the y-axis, the proportion is displayed, while the x-axis displays the location and sample type. Colours annotate the PFAS, and the facet letters on top (A–J) represent the locations.
PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).

Correlation matrix of PFAS concentrations in source materials and eggs
Earlier, in section 3.1, we reported on correlations between PFAS concentrations in eggs and in earthworms for each location (Figure 4) and per PFAS across locations (Figure 5). Figure 12 shows correlations between the PFOS and PFOA concentrations in eggs and in source materials, assessed with Spearman's non-parametric correlation test. This was done for PFOS and PFOA, as these PFAS were the primary contributors to the summed concentrations in eggs and in earthworms (Figures 2 and 3).
PFOS and PFOA concentrations in eggs and in earthworms were correlated ($\rho = 0.75$ and $\rho = 0.84$, respectively), consistent with the findings described above in figures 4 and 5. Furthermore, concentrations of these two PFAS in earthworms and in soil were also correlated ($\rho =$ 0.65 and 0.75, respectively). Correlations of PFOS and PFOA concentrations in water versus eggs, earthworms and soil were lower and not statistically significant. This was also true for PFOS concentrations in eggs versus soil. However, the PFOA concentrations in eggs versus soil were significantly correlated ($\rho = 0.70$). Bedding and miscellaneous was not included in these analyses, because of the variety in sample types.

Figure 12 Spearman's non-parametric correlation matrix for eggs and source materials for PFOS and PFOA concentrations.



This assessment included mean PFAS concentrations in earthworms and soil samples per location. Water samples were included from sources accessible to hens as determined from the questionnaire responses (equal to water sources in Figure 8). One location offered both tap water and rainwater in equal proportions, for which the mean concentration was used. Concentrations below the limit of quantification were included as 0. Bedding and miscellaneous was excluded from these correlation analyses, as that sample category comprised a variety of sample types, with very low PFAS concentrations (below 5 ng/g).
PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).
Statistical significance of Spearman's rho p<0.05 = *, p<0.01 = **.

3.2 Associations between PFAS concentrations in eggs and questionnaire data

Appendix 11 reports on the characteristics of the hens and how they were kept at sixty locations, as reported in the source investigation questionnaire.
Associations between concentrations of PFAS in home-produced eggs from sampling period 1 and various potential determinants of PFAS contamination were assessed by means of multivariable linear regression using the LASSO method (see section 2.6). Table 3 shows the observed associations, in a strict model using the default λ-value with the lowest MSE (λ = 0.26), and a second less strict model for generating hypotheses on associations with the four strongest associations (λ= 0.22). See Appendix 12 for the LASSO regression quality control plots such as the mean cross-validated mean squared error (MSE) for each value of log(λ) and coefficient paths for the input variables.
The strict model yielded two predictive variables in the LASSO model. Lower summed PFAS concentrations in eggs were associated with locations where hens had been kept inside the coop in the preceding six months, and locations with no access to earthworms in the soil (intercept = 1.38, beta coefficient estimate = -0.22 and -0.12 respectively).
The less strict model also yielded the above-mentioned determinants on keeping hens within the coop and no access to earthworms. Additionally, this less strict model yielded that higher summed PFAS concentrations in eggs were associated with locations with older hens (mean age 2.7 to 6 years), and with locations where the area where the hens could roam was unpaved for 77% to 99.5%.

Table 3 Associations between concentrations of PFAS in home-produced eggs from sample period 1, with various potential determinants of PFAS contamination, using multivariable regression with the LASSO method.

Beta coefficient estimates from multivariable linear regression model, using LASSO method with best *λ*

Rank	Variable name: *category*	Beta coefficient estimate
1	Kept indoors/outdoors in past 6 months: *Indoors*	-0.22
2	Access to earthworms: *No*	-0.12

Beta coefficient estimates from multivariable linear regression model, using LASSO method with less strict λ

Rank	Variable name: *category*	Beta coefficient estimate
1	Kept indoors/outdoors in past 6 months: *Indoors*	-0.29
2	Access to earthworms: *No*	-0.19
3	Mean age: *3rd tertile '2.7 to 6 years of age'*	0.01
4	Percentage ground unpaved: *2nd tertile '77% to 99.5%'*	0.05

λ =lambda

Appendices 13 and 14 provide a data file of all variables from the source investigation questionnaire and information on data handling and model entry. They also show supplementary graphs visualising the data distributions and categorisation of the four most predictive variables in the LASSO regression, plus the data distributions and categorisation for all other numerical variables, as well as assigned reference categories.

3.3 PFAS contamination of home-produced eggs throughout the egg-laying season

Dynamics of PFAS contamination in home-produced eggs throughout the egg-laying season were investigated at ten locations, where eggs were collected in the four sampling periods (see Table 1 in section 2.2). At locations I and H, no eggs were collected in sampling period 2 (see section 2.2).

At each location, PFAS concentrations in eggs varied between sampling periods (Figure 13). In addition, the concentrations in the different sampling periods differed per location. The summed concentrations of PFAS in home-produced eggs ranged from

- 0.51 to 16 ng per gram in sampling period 1;
- 1.8 to 53 ng per gram in sampling period 2;
- 0.22 to 24 ng per gram in sampling period 3; and
- 1.0 to 20 ng per gram in sampling period 4 (Figure 13 and Appendix 7).

In sampling period 3 (the first eggs at the start of the laying season), the PFAS concentrations in eggs ranged from 0.22 to 9.8 ng per gram in eggs of hens that had continued producing eggs during the winter and 2.4 to 24 ng per gram in eggs from hens that had stopped their egg production during this period. In both groups, no seasonal effects in PFAS concentrations in eggs were observed (Figure 14) (Kruskal Wallis test, $p>0.05$).

It should be noted that the egg samples in sampling period 4 were analysed with higher LOQs for PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFTrDA, GenX, and ADONA compared to the other three sampling periods (e.g. LOQs for PFOA were 0.4 and 0.025 ng per gram, respectively). This was caused by a signal in the blanks or disturbances in the lower part of the calibration curve. The higher LOQs influenced the summed PFAS concentrations. Since all concentrations below LOQ were set to 0, a higher LOQ would result in a lower summed PFAS concentration. In sampling periods 1 and 2, eggs from four locations, and in sampling period 3, eggs from seven locations had PFOA concentrations between 0.025 and 0.4 ng per gram, which would not have been detected with the LOQ from sampling period 4. However, because PFOS is the most common PFAS in eggs at most locations, and the LOQ of PFOS did not differ across sample periods, we expect that the higher LOQs have not influenced the general trend in summed concentrations across sampling periods.

Figure 13 Sum of PFAS concentrations in home-produced eggs during the four sampling periods for ten locations.

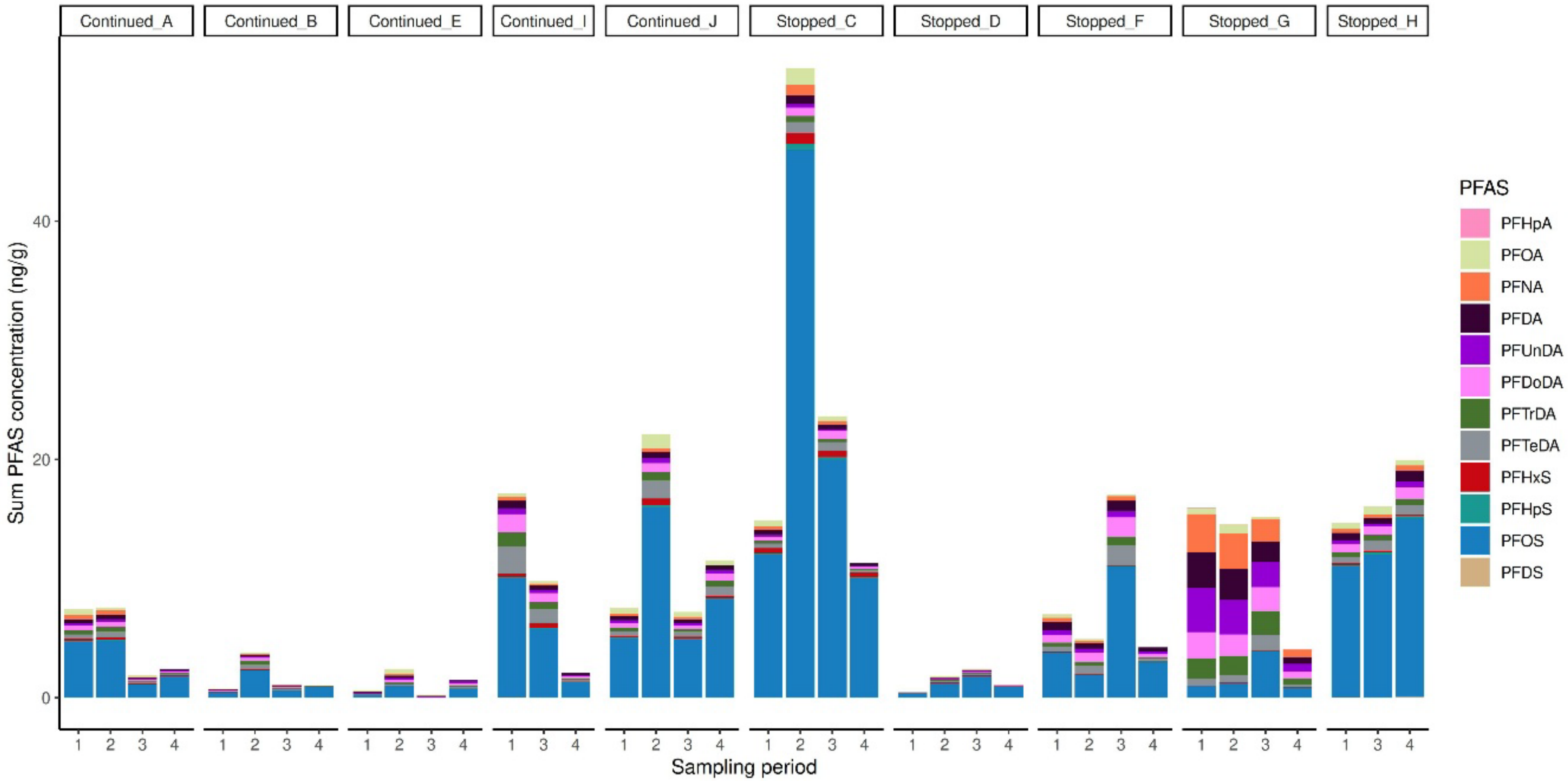


Figure 13 presents the sum PFAS concentration shown on the y-axis in ng/g and the sampling periods (Table 1 in section 2.2) on the x-axis. The facets on top indicate whether hens continued their egg laying during winter (between sampling periods 2 and 3) or stopped (Continued / Stopped) followed by location. Colours represent the PFAS. See also Appendix 7 for the PFAS concentrations in eggs.
PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1)

Figure 14 Sum of PFAS concentrations in home-produced eggs during the four sampling periods at ten locations.

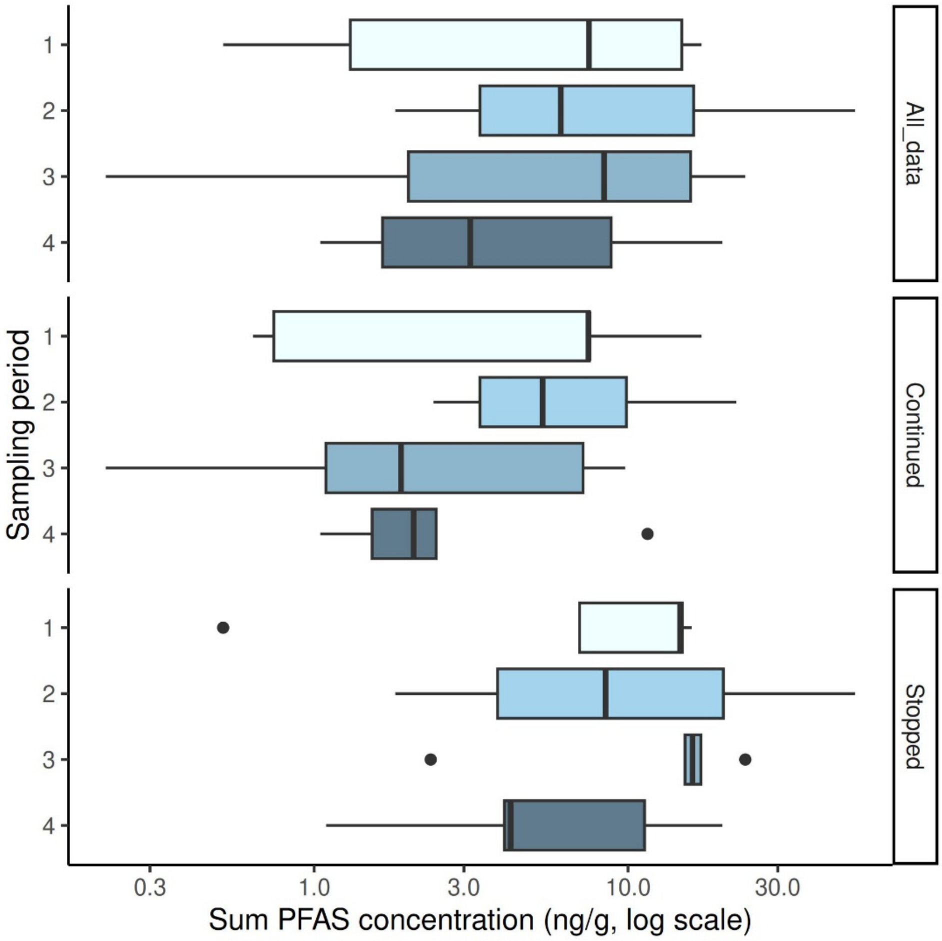


Figure 14 presents boxplots of summed PFAS concentrations in ng/g. On the vertical axis, sampling periods (Table 1 in section 2.2) are displayed (also reflected by shades of blue in boxplots). The facets on the right show whether the plots contain all data (All_data), data from eggs of hens that continued (Continued), or stopped laying eggs during winter (Stopped).

4 Discussion

In this chapter, findings of the current study are compared to available data from literature. Additionally, some limitations and uncertainties in this study are discussed.

4.1 Sources of PFAS contamination in home-produced eggs

The results from PFAS measurements in eggs and in potential source material as well as from the questionnaire data suggest that earthworms are the primary source of PFAS exposure for hens that have access to soil with earthworms. Earthworms contain high concentrations of PFAS and the level and type of PFAS in earthworms correlate with PFAS concentrations measured in eggs. In addition, results from the source investigation questionnaire show that eggs from hens contain lower PFAS concentrations when hens were kept indoors, as well as when they have no access to earthworms, according to the participant. Higher PFAS concentrations were consistently found in eggs from hens with a larger area of unpaved ground to roam on. These three associations found in the source investigation questionnaire indicate that access to earthworms is associated with higher PFAS concentrations in eggs. Earthworms can be an important source of food for hens (Clark et al., 1995). Recently, Lasters et al. (2023) also showed a significant positive relationship between PFOS and PFOA concentrations in eggs and in earthworms, although not with other PFAS. In our study, we found a strong correlation for PFOS and PFOA concentrations between eggs and earthworms. Also PFHxS, PFHpS and PFDS showed a significant positive relationship. Lasters et al. (2023) did not measure PFDS and did not observe PFHpS in home-produced eggs or in earthworms. Consistent with the main finding from our study, a larger ongoing data collection of PFAS in home-produced eggs in the Netherlands, also suggests that access of hens to earthworms is associated with higher PFAS concentrations in eggs.[6] This citizen science website (PFASinkaart.nl) reports preliminary data on a large group of chicken owners in the Netherlands on their website. These owners can report results from commercial PFAS measurements in home-produced eggs. In addition, some additional questions are asked about how chickens are kept.

Next to earthworms, chickens also eat beetles and other small animals (Clark et al., 1995). In this study, a few insects were collected at some locations. Since the amount of insects was small, only one pooled sample was analysed. In this sample, relatively high PFAS concentrations were observed, suggesting that, in addition to earthworms, insects might contribute to the PFAS exposure of hens. However, since only one pooled sample was analysed, no conclusion can be drawn on the contribution of insects to PFAS in home-produced eggs.

Within locations, the pattern of types of PFAS found in soil showed some resemblance to the pattern of PFAS in eggs, although the resemblance was less strong than between earthworms and eggs (Figures 3 and 7). The correlation between soil and eggs for PFOA was significant, but not

[6] Pfasinkaart.nl/analyse (in Dutch)

for PFOS, the predominant PFAS in eggs. However, concentrations of these two PFAS in earthworms and in soil were significantly correlated, indicating that soil is likely to be the source of these two PFAS in earthworms, and thus indirectly in eggs. Differences in PFAS concentrations in soil and in earthworms might be explained by different biota soil accumulation factors (BSAFs).[7] Studies show that the BSAFs for longer-chain PFAS are generally higher than for shorter-chain PFAS (Burkhard and Votava, 2022). This corresponds to the observation in this study and in Nederlof et al. (2025), that long-chain PFAS, such as PFOS and PFOA, were present in higher concentrations in home-produced eggs than short-chain PFAS, such as PFBS and PFPeA. In the current study, BSAFs for PFOS and PFOA were higher than previously used for environmental risk limit derivations (Lijzen et al., 2018; Verbruggen et al., 2020) and the BSAFs reported in a review by Burkhard and Votava (2022) (Appendix 15). These differences may be explained by the weighing of the earthworm. In the current study, the weight of the earthworm was derived after freezing, which corresponds more to dry weight than to wet weight, whereas in other studies wet weight is used for worms.

Information from the source investigation questionnaire, together with the PFAS concentrations in eggs, were positively associated with the age of the hen in the looser LASSO model for hens in the group with ages 2.7–6 years. In contrast to our study, Lasters et al. (2022) observed that eggs from young hens (<1 year) had higher PFOA concentrations than eggs from older hens, and that there was no relationship between age and PFOS concentrations in eggs. However, the ‘older’ hens in that study were still relatively young (2.8 ± 1 (SD) years), whereas hens in private gardens can continue laying eggs until they are 8 years of age (Ali, 2020). Hens in our study were between 0.5 and 6 years old (Appendix 11). Higher PFAS concentrations in eggs from older hens can be explained by a reduction in egg production, (Joyner et al., 1987). PFAS is excreted from hens through egg laying, and the PFAS concentration in eggs is related to the exposure to PFAS during a steady-state situation (FO, 2023; Kowalczyk et al., 2020; Wilson et al., 2021). With a constant exposure to PFAS, the PFAS concentration in the eggs could hence increase with lower egg production (FO, 2023). Lasters et al. (2022) also use this idea to explain the higher PFAS concentrations in young hens in their study. Young hens had only had one egg-laying cycle and still a high PFAS body burden from maternal transfer. Another factor why eggs from older hens could contain more PFAS is that yolk weight increases with the age of the hen (Suk and Park, 2001). Since PFOS and PFOA primarily accumulate in the yolk, larger egg yolks could be a cause of higher PFAS concentrations in eggs (Wang et al., 2019).

Chicken feed was not tested for PFAS in this study, because in a recent study by Arcadis no PFAS was found in feed (Arcadis, 2024). Correspondingly, our questionnaire showed no correlation between feed and PFAS concentrations in eggs. Previously, Lasters et al. (2022) observed higher PFOS and PFOA concentrations in eggs from hens that

[7] BSAF (Biota Soil Accumulation Factor) is a measure of the extent to which a chemical accumulates in organisms (biota), such as plants or animals, relative to its concentration in the soil where the organism lives. It thus indicates how efficiently a substance is taken up from the soil by living organisms.

were fed an obligate diet of kitchen leftovers. In our study, none of the participants fed their hens such a diet, although some participants occasionally fed their hens some kitchen leftovers. Moreover, volunteers in the study from Lasters et al. (2022) lived close to a fluorochemical plant that has emitted PFOS, and many cultivated their own plant crops. It was shown that crops grown near this chemical plant can contain elevated PFAS levels (Consortium UAntwerpen, VITO, PIH, UHasselt and VUB, 2023). From our questionnaire, it cannot be deduced whether the kitchen leftovers were homegrown.

Previously it has been suggested that fishmeal added to the organic chicken feed could explain the high PFAS concentrations found in the home-produced eggs, (Granby et al., 2024). In our study, no participants reported feeding their chickens fishmeal.

In this study, little or no PFAS were observed in bedding and scraping from the chicken coop, indicating that this is not likely to be an important source of PFAS in home-produced eggs. Fernandes et al. (2023) reported that exposure to PFAS via cardboard and dried pulp bedding could result in higher PFOS concentrations in eggs, whereas this was not the case for wood shavings. At the ten follow-up locations of our study, no cardboard or pulp bedding was observed in the surroundings of the hens.

The locations of our study were selected throughout the Netherlands, and hence this study was not designed to focus on possible PFAS contamination from region-specific sources such as industry. Industry might be a cause of locally higher PFAS concentrations in home-produced eggs. We have shown before that PFOA concentrations were higher in eggs in the vicinity of Chemours than in the rest of the Netherlands (Nederlof et al., 2025). Furthermore, elevated PFOS concentrations were detected around a fluorochemical plant in Antwerp. PFOS concentrations steeply declined with increasing distance from the fluorochemical plant, (Lasters et al., 2022).

PFAS precursors were also measured in this study. When these precursors are ingested by hens, they can end up as PFAS in eggs (Kowalczyk et al., 2020). Since PFOS is the most abundant PFAS in most egg samples, PFOS precursors were analysed in source material. Almost no PFOS precursors were detected in soil, water and bedding and coop material, and some were detected in earthworms, indicating that PFOS precursors are not likely to be an important source of PFOS in home-produced eggs.

4.2 Seasonality of PFAS contamination in home-produced eggs

We hypothesised that there are seasonal differences in PFAS concentrations in home-produced eggs. As described above, PFAS concentrations per egg could increase with lower laying frequencies (see section 4.1). Hens may reduce or stop laying eggs when they are moulting. Moulting is a natural process in which old feathers are replaced by new ones, usually lasting one to two months and mainly occurring in autumn. During this period, chickens often stop laying eggs as they need their energy for growing new feathers. Also, when the number of daylight hours drops below twelve per day, most hens will lay fewer eggs or may even stop laying altogether. Their bodies respond to the reduced light and produce less hormones required for egg production. Chicken owners can stop this effect or make it less pronounced by using artificial lighting to extend the daylight hours.

In this study, with four sampling periods at ten follow-up locations across the Netherlands (see Figure 1 in section 2.1), sufficient insight could be gained into possible seasonality of PFAS concentrations in home-produced eggs. At all locations, these concentrations varied at different times of the year (see Figure 13 in section 3.3). At some locations, PFAS concentrations were lower in summer. Furthermore, the first eggs laid after winter did not contain significantly higher PFAS concentrations than eggs from other sampling periods, even at locations where chicken owners reported that their hens had stopped laying eggs during winter. Thus, we did not find clear support for our hypothesis that concentrations of PFAS are strongly affected by the annual cycle of decreased or paused egg production in chickens. Other factors also seem to play a role in the fluctuation of PFAS concentrations in home-produced eggs during the year. In addition, the large fluctuations show that a single measurement is only a snapshot and provides limited information about PFAS concentrations in home-produced eggs during the year.

4.3 Limitations and uncertainties

To calculate the summed PFAS concentrations, the PFAS concentrations that were reported as '< LOQ' were assumed to equal zero (see Table 2 in section 2.5). This assumption may have underestimated the actual summed concentrations because the unquantified ('< LOQ') PFAS may actually be present in the samples. However, the PFAS concentrations that could be quantified in eggs were high compared to the LOQ in both eggs and the source materials. Therefore, it is expected that this did not influence the conclusions of this study.

At ten follow-up locations, source material was analysed for PFAS. Some relevant sources may have been overlooked due to the modest number of investigated locations. Possible sources of PFAS contamination have been analysed from various materials. As PFAS analyses in other matrices than food, water, and soil were not yet fully developed and validated, not all PFAS could be measured in all materials. diSAmPAP could not be measured with WFSR's methods of analysis, due to its physical-chemical properties. PFBA could not be determined in any sample because of high background levels.

It was decided to use the PFAS concentrations in soil that were not normalised to organic carbon (section 2.5). The normalised concentrations are only reported in Appendices 3 and 10 and were not used in the correlation matrix (section 3.1). In earlier RIVM evaluations in the context of deriving environmental risk limits for PFOA and PFOS in soil, it was also decided to refrain from normalisation (Lijzen et al., 2018; Verbruggen et al., 2020). In these publications, for both PFOS and PFOA, the variability in bioaccumulation from soil to earthworms increased slightly, but not significantly, after normalisation. Also, in the current study, little difference was seen between normalised and non-normalised soil PFAS concentrations, indicating that this has very likely not affected our conclusions.

Eggs from multiple hens were pooled per location per sampling period, so no distinction could be made as to which egg comes from which hen. Therefore, also in the questionnaire, an average age and laying frequency was determined per location. This may have diluted some of the effects observed in this study.

Despite some limitations and uncertainties, our study provides clear results from PFAS measurements, as well as from questionnaire associations. Therefore, we conclude that the study design was sufficient to provide a good indication of the most important source(s) of PFAS contamination in home-produced eggs in the Netherlands.

5 Conclusion

In early 2025, RIVM reported that home-produced eggs throughout the Netherlands can contain high concentrations of PFAS, more than commercial eggs. Therefore, the RIVM advised not to consume home-produced eggs (Nederlof et al., 2025).

The current study examined potential sources of elevated PFAS concentrations in home-produced eggs, and showed that the consumption of earthworms by hens may be the most important source. One small mixed sample of insects also contained high PFAS concentrations. However, since only one sample was analysed, no conclusion could be drawn as to whether other invertebrates, such as insects, may be an important source of PFAS in home-produced eggs.

At all examined locations, earthworms contained high PFAS concentrations and these concentrations were correlated with PFAS concentrations in the respective eggs. Furthermore, the pattern of PFAS in earthworms closely matched that in eggs. Eggs from hens that had been kept indoors for the past six months or had no access to earthworms had lower PFAS concentrations. Furthermore, hens that had greater access to unpaved ground had higher PFAS concentrations in their eggs. Concentrations of PFAS in soil were correlated to PFAS levels in worms. It is plausible that PFAS from soil accumulate in earthworms.

Concentrations of PFAS in soil were much lower than in earthworms and correlated less with PFAS in eggs. Therefore, soil as a direct source of PFAS in eggs is likely to be limited. Also, few PFAS were reported in water, bedding, and coop material. However, these concentrations were too low to explain the high PFAS concentrations in home-produced eggs.

PFAS concentrations in home-produced eggs varied during the year per location. However, no seasonal differences were observed and thus no clear evidence that concentrations of PFAS are affected by the annual cycle of egg production. The variation between the different sampling periods also shows that a single concentration of PFAS in home-produced eggs does not provide reliable information about the safety of consuming the eggs.

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Appendix 1 Names and abbreviations of analysed PFAS compounds

Abbreviation	**Compound names**	**Measured in**				
		Eggs	**Earthworms**	**Soil**	**Water**	**Bedding and coop material**
Sulfonic acids						
PFBS	Perfluorobutane sulfonic acid	X	X	X	X	X
PFPeS	Perfluoropentane sulfonic acid		X	X	X	X
PFHxS	Perfluorohexane sulfonic acid	X	X	X	X	X
PFHpS	Perfluoroheptane sulfonic acid	X	X	X	X	X
PFOS	Perfluorooctane sulfonic acid		X	X	X	X
PFNS	Perfluorononane sulfonic acid	X	X	X	X	X
PFDS	Perfluorodecane sulfonic acid	X	X	X	X	X
PFUnDS	Perfluoroundecane sulfonic acid		X	X	X	X
PFDoDS	Perfluorododecane sulfonic acid		X	X	X	X
PFTrDS	Perfluorotridecane sulfonic acid		X	X	X	X
Carboxylic acids						
PFBA	Perfluorobutanoic acid	X				
PFPeA	Perfluoropentanoic acid	X	X	X	X	X
PFHxA	Perfluorohexanoic acid	X	X	X	X	X
PFHpA	Perfluoroheptanoic acid	X	X	X	X	X
PFOA	Perfluorooctanoic acid	X	X	X	X	X
PFNA	Perfluorononanoic acid	X	X	X	X	X
PFDA	Perfluorodecanoic acid	X	X	X	X	X
PFUnDA	Perfluoroundecanoic acid	X	X	X	X	X
PFDoDA	Perfluorododecanoic acid	X	X	X	X	X
PFTrDA	Perfluorotridecanoic acid	X	X	X	X	X
PFTeDA	Perfluorotetradecanoic acid	X	X	X	X	X

Abbreviation	Compound names	Measured in				
		Eggs	Earthworms	Soil	Water	Bedding and coop material
Ether carboxylic acids						
HFPO-DA (GenX)	9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	X	X	X	X	X
ADONA	Ammonium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	X	X	X	X	X
n:2 Fluorotelomer sulfonic acids						
4:2 FTS	4:2 Fluorotelomer sulfonic acid		X	X	X	X
6:2 FTS	6:2 Fluorotelomer sulfonic acid		X	X	X	X
8:2 FTS	8:2 Fluorotelomer sulfonic acid		X	X	X	X
10:2 FTS	10:2 Fluorotelomer sulfonic acid		X	X	X	X
Precursors of PFOS						
diSAmPAP	di(N-ethyl-2-perfluorooctane sulfonamido ethyl) phosphate		X	X	X	X
MeFOSA	Methylperfluorooctanesulfonamide		X	X	X	X
EtFOSA	Ethylperfluorooctanesulfonamide		X	X	X	X
FOSAA	Perfluorooctane sulfonamidoacetic acid		X	X	X	X
MeFOSAA	2-(N-Methylperfluorooctanesulfonamido)acetic acid		X	X	X	X
EtFOSAA	2-(N-Ethylperfluorooctanesulfonamido)acetic acid		X	X	X	X
MeFOSE	Methylperfluorooctanesulfonamidoethanol		X	X	X	X
EtFOSE	Ethylperfluorooctanesulfonamidoethanol		X	X	X	X
PFOSA	Perfluoro-octane sulfonamide		X	X	X	X
Other PFASs						
PFECHS	Perfluoro-4-ethylcyclohexane sulfonate		X	X	X	X
8Cl-PFOS	8-chloroperfluoro-1-octanesulfonic acid		X	X	X	X
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonate				X	X
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonate				X	X

Appendix 2 PFAS concentrations (in ng per g) in earthworms and insects per location

https://www.rivm.nl/bibliotheek/rapporten/2025-0170.xlsx

Appendix 3 PFAS concentrations (in ng per g) in soil per location

https://www.rivm.nl/bibliotheek/rapporten/2025-0170.xlsx

Appendix 4 PFAS concentrations (in µg per L) in water per location

https://www.rivm.nl/bibliotheek/rapporten/2025-0170.xlsx

Appendix 5 Source investigation questionnaire

☐ = answer category, multiple answers possible
O = answer category, one answer possible
……… = open text answer

Chapter 1: General questions

Through these questions, we would like to gather some general information about your hens. We also ask you to provide your details again so that we know which eggs belong to which questionnaire.

Question 1:
What is your place (municipality) of residence?
………………………..

Question 2:
What is your email address?
………………………..

Question 3:
How many hens (not chicks and roosters) do you have?
………………………..

Question 4:
What breed are your egg-laying hens (excluding cockerels or chicks)?

- ☐ The Brahma
- ☐ The Barnevelder
- ☐ The Silkie
- ☐ The Dutch Bantam
- ☐ The Serama
- ☐ The Kraaikop
- ☐ The Welsumer
- ☐ The Mechelse (Malines)
- ☐ The Leghorn
- ☐ The Wyandotte Bantam
- ☐ The Orpington
- ☐ The Araucana
- ☐ The Brabanter
- ☐ The Sussex
- ☐ The Lakenvelder
- ☐ Rescued white layer hen
- ☐ Rescued brown layer hen
- ☐ Other: ………………………..

Question 5:
How old are your hens?
Please indicate in years and round down. For example, if your hen is 2.5 years old, enter 2. If you do not know the exact age, please estimate how old your hens are. Please specify the age for each hen separately.

- ☐ Hen 1: ………………………..
- ☐ Hen 2: ………………………..

- ☐ Hen 3: ………………………..
- ☐ Hen 4: ………………………..
- ☐ Hen 5: ………………………..
- ☐ Hen 6: ………………………..
- ☐ Hen 7: ………………………..
- ☐ Hen 8: ………………………..
- ☐ Hen 9: ………………………..

Question 6:
How long have you had your hens?
Please indicate in years and round down. For example, if you have had your hen for 2.5 years, enter 2.
- ☐ Hen 1: ………………………..
- ☐ Hen 2: ………………………..
- ☐ Hen 3: ………………………..
- ☐ Hen 4: ………………………..
- ☐ Hen 5: ………………………..
- ☐ Hen 6: ………………………..
- ☐ Hen 7: ………………………..
- ☐ Hen 8: ………………………..
- ☐ Hen 9: ………………………..

Question 7:
Where do your hens come from?
- ☐ From the local area (within approximately 5 kilometres of your home)
- ☐ Wholesale
- ☐ I don’t know
- ☐ From outside the local area, namely from: ………………………..

Question 8:
Have your older hens moulted yet?
- O Yes
- O No

if question 8=Yes
Question 8a: You have indicated that your hens have moulted. Could you specify which hens have moulted?
- ☐ Hen 1
- ☐ Hen 2
- ☐ Hen 3
- ☐ Hen 4
- ☐ Hen 5
- ☐ Hen 6
- ☐ Hen 7
- ☐ Hen 8
- ☐ Hen 9

Question 9: When did your hens last moult?
- O September 2024
- O August 2024
- O July 2024
- O June 2024
- O May 2024

- O April 2024
- O March 2024
- O February 2024
- O January 2024
- O December 2023
- O November 2023
- O October 2023

Question 10:
Do you provide your hens with supplemental lighting (using lamps)?
- O Yes
- O No

Chapter 2: Egg Laying

In this chapter, we would like to learn more about the egg-laying frequency of your hens.

Question 11:
Could you indicate how many eggs your hens lay on average per week, based on the times you have collected eggs?
If possible, please enter the data for each hen separately. If you only know the total number of eggs, please divide this by the number of hens.
Hen 1: ....
Hen 2: ....
Hen 3: ....
Hen 4: ....
Hen 5: ....
Hen 6: ....
Hen 7: ....
Hen 8: ....
Hen 9: ....

Question 12:
Do your hens lay eggs all year round?
- O Yes
- O No

Question 13:
Are your hens currently laying fewer eggs than usual?
- O Yes
- O No

Chapter 3: Feed

In this chapter, we would like to know what your hens eat. This includes their main feed, as well as any snacks you occasionally give them.

Question 14:
What type of chicken feed do you use?
(You may select up to 4 options)
- ☐ Complete layer pellets
- ☐ Complete layer mash
- ☐ Premium mix (a blend of 2 or 3 seeds)

- ☐ Mixed grains as a complete feed
- ☐ Dried insects as the main ingredient
- ☐ Kitchen scraps
- ☐ Bread
- ☐ Other: ………………………..

Question 15:
What brand of chicken feed do you use?
Please specify the type and brand. (For example: layer pellets AR 20 kg or mixed grains from Farmer Janssen in Amsterdam)
………………………..

Question 16:
What type of packaging does the chicken feed come in?
- O Plastic
- O Paper
- O None
- O Other, namely: ………………………..

Question 17:
Could you upload a photo or photos showing the brand and ingredients of the feed?
………………………..

Question 18:
Do you feed your hens from a feeder or do they peck the feed from the ground?
- ☐ Feeder
- ☐ Ground
- ☐ Dish/bowl on the ground
- ☐ Other, namely: ………………………..

Question 19:
What material are the feeder or the dishes/bowls made of?
If you do not use a feeder or dishes/bowls, please enter 'not applicable'.
- ☐ Metal
- ☐ Wood
- ☐ Plastic
- ☐ Not applicable
- ☐ Other, namely ………………………..

Question 20:
Do you give your hens snacks?
- O Yes
- O No

if question 20=Yes
You have indicated that you give your hens snacks.
What snacks do you give them?
- ☐ Dried insects (more than half a teacup per day)
- ☐ Dried insects (less than half a teacup per day)
- ☐ Mixed grains
- ☐ Other, namely: ………………………..

Question 21:
Did you change the chicken feed in the past six months?
- O Yes
- O No

if question 21=Yes
What type of chicken feed did you use previously?
(You may select up to 4 options)
- ☐ Complete layer pellets
- ☐ Complete layer mash
- ☐ Premium mix (a blend of 2 or 3 seeds)
- ☐ Mixed grains as a complete feed
- ☐ Dried insects as the main ingredient
- ☐ Kitchen scraps
- ☐ Bread
- ☐ Other: ……………………….

Question 22:
Do you use special (layer) feed to get your hens to lay eggs?
- O Yes
- O No

if question 22=Yes
Type of feed: ……………………….

In which month(s) do you provide this feed?
- ☐ January
- ☐ February
- ☐ March
- ☐ April
- ☐ May
- ☐ June
- ☐ July
- ☐ August
- ☐ September
- ☐ October
- ☐ November
- ☐ December

Question 23:
Do you give your hens different feed when they are moulting?
- O Yes
- O No

if question 23=Yes
What type of different feed do you give when your hens are moulting?
……………………….

Question 24:
Do you supplement your hens' diet with fishmeal yourself?
- O Yes
- O No

if question 24=Yes

How many times per week do you feed fishmeal?

…………………………

What brand of fishmeal do you use?

…………………………

What type of packaging does the fishmeal come in?

O plastic
O paper
O none
O other, namely: …………………………

Question 25:

Do you feed fish waste to your hens?

O Yes
O No

if question 25=Yes

How many times per week do you feed fish waste to your hens?

…………………………

Question 26:

Do you feed (leftover) meat to your hens?

O Yes
O No

if question 26=Yes

How many times per week do your hens receive (leftover) meat?

…………………………

Question 27:

Do you use poultry grit/oyster shell grit/gizzard stones?

O Yes
O No

if question 27=Yes

Which brand of poultry grit/oyster shell grit/gizzard stones do you use?

…………………………

In what type of packaging do you buy these poultry grit/oyster shell grit/gizzard stones?

O paper
O plastic
O other, namely: …………………………

Question 28:

Do you also feed your hens calcium?

O Yes
O No

Question 29:
Do you feed green waste to your hens?

- ☐ no
- ☐ grass
- ☐ leaves
- ☐ kitchen scraps
- ☐ other, namely: ……………………………

Question 30:
Do you feed any other supplementary feed?
(You may select up to 3 options)

- ☐ cat food
- ☐ blood meal
- ☐ dog sausage
- ☐ animal meal
- ☐ other, namely: ……………………………

Question 31:
What type of water do you give your hens?

- ☐ tap water
- ☐ rainwater
- ☐ pond water
- ☐ ditch water
- ☐ groundwater

In the follow-up question, you can indicate what percentage this is (for example, 100% if only tap water is given, or 60% tap water and 40% ditch water—the total must always add up to 100%. If you do not know the exact percentages, please make an estimate).

You indicated that your hens receive tap water. What percentage of their total drinking water does this represent? (e.g. 20%)
……………………………

You indicated that your hens receive rainwater. What percentage of their total drinking water does this represent? (e.g. 20%)
……………………………

You indicated that your hens receive pond water. What percentage of their total drinking water does this represent? (e.g. 20%)
……………………………

You indicated that your hens receive ditch water. What percentage of their total drinking water does this represent? (e.g.20%)
……………………………

You indicated that your hens receive groundwater. What percentage of their total drinking water does this represent? (e.g. 20%)
……………………………

Chapter 4: Soil

The following questions are about all the soil your hens regularly have access to. This includes the soil under the chicken run as well as any surrounding ground where your hens roam.
We will also ask you about other elements near the chicken coop.

Question 32:
Where does the soil your hens walk on come from?
- ☐ original (garden) soil
- ☐ soil has been removed
- ☐ soil has been added
- ☐ other, namely: ....

Question 33:
Do you ever add fresh soil (e.g. turf) to the area where your hens walk?
- O Yes
- O No

Question 34:
Do you work the soil where your hens walk? By 'work' we mean digging, turning, or similar activities.
- O Yes
- O No

if question 34=Yes
Can you explain what you have done to the soil in your garden?
....

Question 35:
How many hours per week do your hens spend on unpaved (natural) ground?
(For this answer, please provide an average number of hours per week. For example: 4 days x 6 hours = 24 and 3 days x 5 hours = 15. 24 + 15 = 39 hours.)
....

Question 36:
How many square metres (m²) of the area where your hens roam is paved and covered?
(By m² we mean the length x width of your garden/chicken house or run. For example: 4 x 5 metres = 20m².)
....

Question 37:
How many square metres (m²) of the area where your hens roam is paved and not covered?
(Same calculation as above.)
....

Question 38:
How many square metres (m²) of the area where your hens roam is unpaved and covered?
(Same calculation as above.)

Question 39:
How many square metres (m²) of the area where your hens roam is unpaved and not covered?
(Same calculation as above.)
…………………………

Question 40:
Is the unpaved ground your hens scratch on compacted/hard or loose?
- O Compacted/hard
- O Loose
- O A combination of compacted/hard and loose

if question 40= Loose OR A combination of compacted/hard and loose
How many hours per day do the hens spend on loose soil?
…………………………

Question 41:
In your opinion, can your hens access the earthworms in the soil?
- O Yes
- O No
- O I don't know

if question 41=Yes
Do your hens eat these earthworms?
- O Yes
- O No
- O I don't know

Question 42:
Do your hens also eat other creatures (besides earthworms)?
- O Yes
- O No
- O I don't know

if question 42=Yes
You indicated that your hens also eat other creatures. Which ones do they eat?
You can select more than one answer.
- ☐ Spiders
- ☐ Flies
- ☐ Garden snails
- ☐ Mice
- ☐ Young birds
- ☐ Cadavers
- ☐ Other, namely: …………………………

Question 43:
Do you provide enrichment materials for your hens, such as toys or small bridges?
- O Yes
- O No

if question 43=Yes

You indicated that you provide enrichment materials for your hens. What are these made of?

- ☐ Wood
- ☐ Plastic
- ☐ Metal
- ☐ Other, namely: ………………………..

Question 44:

Which of the following items are present in the area where your hens roam? The hens do not need to have access to them.

- ☐ Parasol
- ☐ Sunshade
- ☐ Tent
- ☐ Solar panels
- ☐ Carpet
- ☐ Other outdoor textiles (for example, cushions)
- ☐ Plastic screening net
- ☐ None of the above
- ☐ Other, namely: ………………………..

Question 45:

To your knowledge, has fire-fighting foam ever been used in or around your house? This may also have been a long time ago.

- O Yes
- O No

if question 45=Yes

Can you give an approximate year when fire-fighting foam was used around your house?

………………………..

Question 46:

Do you use products such as lubricants or water-repellent sprays for clothing or parasols near (within 10 metres of) the areas where your hens roam?

- O Yes
- O No

Chapter 5: The chickencoop and the run

With the following questions, we would like to gather more information about the coop where your hens stay and, if you have one, the run where your hens roam.

Question 47:

For how many years has the chicken coop or run been in its current location? *(Please round up, so 1.5 years becomes 2 years)*

………………………..

Question 48:

Do you have a run?

- O Yes
- O No

if question 48=Yes

What is the size of the run (in m²)?

…………………………

What material is the run made of?

- ☐ Wood
- ☐ Iron
- ☐ Plastic
- ☐ Corrugated sheeting
- ☐ Other, namely: …………………………

Question 49:

Is your chicken coop coated?

- O Yes
- O No

Question 50:

Do you use bedding material in the coop or in the run?

- ☐ No
- ☐ Wood
- ☐ Paper
- ☐ Wood shavings
- ☐ Straw
- ☐ Carpet
- ☐ Sand
- ☐ Cat litter
- ☐ Other, namely: …………………………

Question 51:

Do your hens scratch for food directly around the house, or further away?

- O Directly around the house
- O Further away

if question 51=Further away

Can you indicate how far from your house the hens roam?

…………………………

Can you upload photos of the coop, the run, and the area(s) where the hens roam?

…………………………

Question 52:

In addition to the previous questions, could you please describe in more detail how your hens are kept? What does their accommodation look like, and where are they able to roam?

…………………………

Question 53:

Have your hens spent most of the past six months indoors or outdoors?

- O Indoors
- O Outdoors
- O Both

Question 54:
Do you use cleaning products to clean the run or the coop?
- O Yes
- O No

if question 54=Yes
Can you provide the brand and type of cleaning product you use?
…………………………

Where in the coop/run do you use these cleaning products?
…………………………

Question 55:
Do you use any products to make the run or coop waterproof (impregnating)?
- O Yes
- O No

if question 55=Yes
What brand and type do you use to make the run or coop waterproof?
…………………………

Where do you apply this product in the run or coop?
…………………………

Question 56:
Do your hens peck at anything other than their feed, the ground, and water (either inside or outside the run)?
- O Yes
- O No

if question 56=Yes
What else do your hens peck at? (You can select more than one answer)
- ☐ Wood from the run
- ☐ Insulation material
- ☐ Chicken wire
- ☐ Other, namely: …………………………

Chapter 6: Medication

The following questions are about the current use of medication and/or vitamins for your hens.

Question 57:
Do your hens receive any medication and/or vitamins?
- O Yes
- O No

if question 57=Yes

Since what year have you been giving medication and/or vitamins?

………………………

Can you specify which medication or vitamins you use and how often they are given?

………………………

Question 58:

Do you use any anti-lice or mite treatments?

O Yes

O No

if question 58=Yes

Where do you apply the anti-lice/mite treatment?

☐ Coop

☐ Chickens

☐ Feed

☐ Water

Other, namely: ………………………

When was the last time you used an anti-lice/mite product?

Please select the month and enter the year (e.g. 2020).

☐ January

☐ February

☐ March

☐ April

☐ May

☐ June

☐ July

☐ August

☐ September

☐ October

☐ November

☐ December

☐ Year: ………………………

Question 59:

Are there any other details you think may be of value to our research?

Please note: there is limited space to enter your response.

………………………

Question 60:

May we contact you if we have any further questions?

O Yes

O No

if question 60=Yes

How may we contact you?

☐ Telephone

☐ Email

Appendix 6 LASSO regression methodology

The LASSO method (Least Absolute Shrinkage and Selection Operator) is particularly useful when dealing with high-dimensional datasets where multicollinearity is present.[8,9] It provides a regression analysis method that performs both variable selection and regularisation to enhance the prediction accuracy and interpretability of the resulting statistical model. To yield model outcomes with relevant associations, the LASSO regression requires definition of a lambda (λ) value in the model setting. The default method of determining a suitable λ for LASSO regression is the λ-value that produces the lowest test mean squared error (MSE), as identified through 10-fold cross-validation. The lowest MSE provides the best fit between the observed data and the predictions made by the LASSO regression model.[10] However, for the purpose of this study, besides the best (lowest) λ, a less strict λ-value was also applied, to create more space for results to appear in the model outcome for the purpose of generating hypotheses.

[8] https://www.statology.org/lasso-regression/
[9] https://www.statology.org/lasso-regression-in-r/
[10] https://www.statology.org/k-fold-cross-validation

Appendix 7 PFAS concentrations (in ng per gram) in home-produced eggs per location

https://www.rivm.nl/bibliotheek/rapporten/2025-0170.xlsx

Appendix 8 PFAS concentrations (in ng per g) in bedding and miscellaneous per location

https://www.rivm.nl/bibliotheek/rapporten/2025-0170.xlsx

Appendix 9 The main contributing PFAS per location (A to J), expressed as percentage contribution of individual PFAS to the summed concentrations of PFAS.

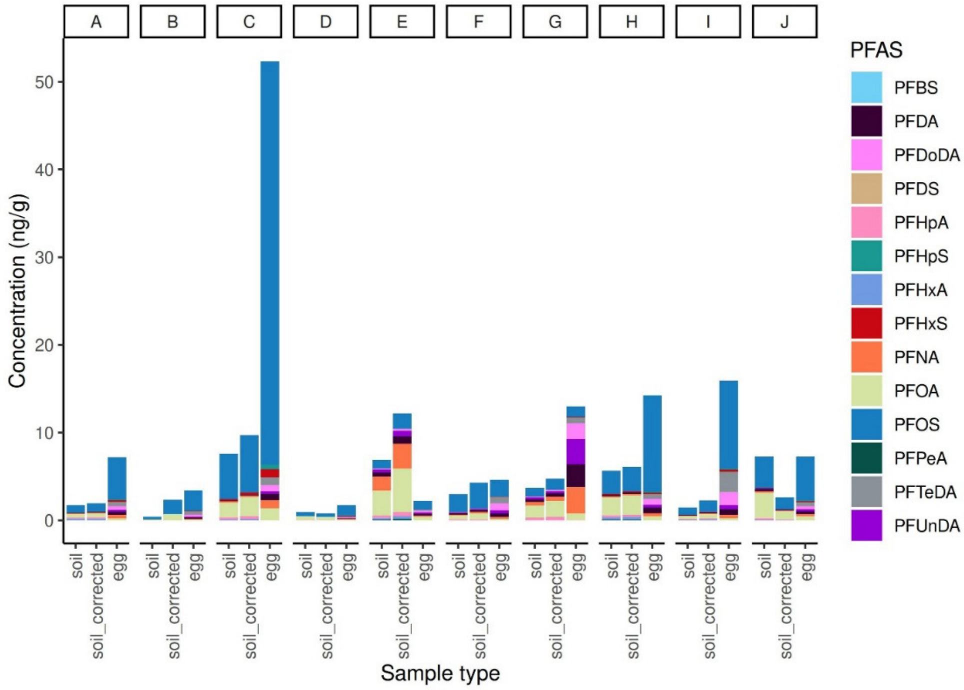
Location & source	**Summed concen-tration (ng/gram)**	**Top 3 PFAS* (contribution to the summed concentration in percentage)**		
		1st contributor	**2nd contributor**	**3rd contributor**
Location A				
eggs	7.2	PFOS (67%)	PFTeDA (7%)	PFDoDA (6%)
earthworms	16	PFOS (46%)	PFOA (13%)	PFHpA (8%)
soil	1.7	PFOS (49%)	PFOA (24%)	PFHxA (10%)
tap water	0	-	-	-
Location B				
eggs	3.4	PFOS (67%)	PFTeDA (12%)	PFDoDA (6%)
earthworms	16	PFOS (67%)	PFTeDA (18%)	PFDoDA (6%)
soil	0.4	PFOS (76%)	PFOA (24%)	-
tap water	0	-	-	-
Location C				
eggs	52	PFOS (88%)	PFOA (3%)	PFHxS (2%)
earthworms	181	PFOS (68%)	PFOA (18%)	PFHxS (2%)
soil	7.6	PFOS (68%)	PFOA (22%)	PFHpA (3%)
ground water	0.19	PFOA (52%)	PFHxS (16%)	PFOS (12%)
rain water	0.01	PFOS (69%)	PFHxS (10%)	PFHpA (9%)
Location D				
eggs	1.7	PFOS (69%)	PFOA (7%)	PFTeDA (6%)
earthworms	5.1	PFOS (53%)	PFOA (15%)	PFTeDA (9%)
soil	0.94	PFOS (52%)	PFOA (44%)	PFHpA (4%)
tap water	0	-	-	-
Location E				
eggs	2.2	PFOS (45%)	PFOA (19%)	PFDA (9%)
earthworms	54	PFOS (22%)	PFOA (18%)	PFTeDA (16%)
soil	6.9	PFOA (41%)	PFNA (23%)	PFOS (14%)
tap water	0	-	-	-
Location F				
eggs	4.6	PFOS (41%)	PFDoDA (17%)	PFTeDA (16%)
worms	15	PFOS (58%)	PFTeDA (19%)	PFDoDA (6%)
soil	3	PFOS (69%)	PFOA (15%)	PFDA (5%)
tap water	<0.001	PFOS (100%)	-	-
Location G				
eggs	13	PFNA (23%)	PFUnDA (22%)	PFDA (20%)
earthworms	27	PFOS (23%)	PFDoDA (16%)	PFUnDA (16%)

Location & source	Summed concen-tration (ng/gram)	Top 3 PFAS* (contribution to the summed concentration in percentage)		
		1st contributor	2nd contributor	3rd contributor
soil	3.7	PFOA (38%)	PFOS (27%)	PFNA (11%)
tap water	<0.001	PFBS (32%)	PFHxA (27%)	PFHxS (22%)
Location H				
eggs	14	PFOS (77%)	PFDoDA (5%)	PFDA (4%)
earthworms	264	PFOS (63%)	PFOA (18%)	PFTeDA (5%)
soil	5.7	PFOS (47%)	PFOA (36%)	PFHxA (5%)
tap water	0	-	-	-
Location I				
eggs	16	PFOS (63%)	PFTeDA (14%)	PFDoDA (9%)
earthworms	70	PFOS (64%)	PFHxS (8%)	PFTeDA (7%)
soil	1.5	PFOS (57%)	PFOA (21%)	PFHpA (5%)
tap water	0	-	-	-
Location J				
eggs	7.2	PFOS (69%)	PFOA (6%)	PFTeDA (6%)
earthworms	113	PFOS (65%)	PFOA (11%)	PFTeDA (7%)
soil	7.3	PFOS (49%)	PFOA (40%)	PFNA (3%)
tap water	0	-	-	-
rain water	0.01	PFOA (42%)	PFHxA (27%)	PFHpA (22%)
surface water	0.03	PFOA (31%)	PFHxA (20%)	PFBS (16%)

Proportion of PFAS in eggs and potential sources of contamination at the ten follow-up locations. This table does not include the sample category bedding and miscellaneous, because that category comprised highly heterogeneous materials with very low PFAS concentrations (below 5 ng/g). Section 2.5 describes the summation of PFAS concentrations.
* PFAS: Per- and polyfluoroalkyl substances. The names of PFAS compounds can be found in Appendix 1.

Appendix 10 PFAS concentrations in soil, uncorrected and corrected for organic matter

Figure A1 Concentrations of PFAS in nanogram (ng) per gram in soil (mean concentrations of soil of different PFAS; see section 2.4), both uncorrected and corrected for organic carbon, and eggs at the ten follow-up locations.



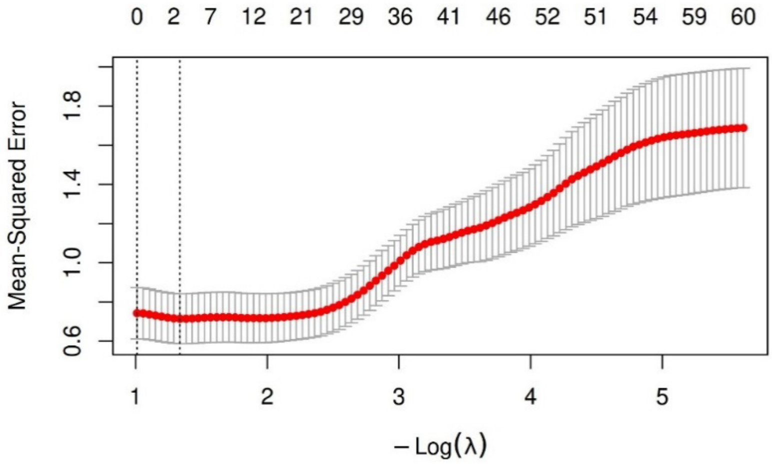
PFAS are depicted when analysed and reported in soil as well as in eggs, with concentrations above the limit of quantification. On the y-axis PFAS concentration in nanogram per gram is displayed, while the x-axis displays the sample type. Soil is uncorrected for organic matter, soil corrected is corrected for organic matter. Colours annotate the PFAS and the facet letters on top (A–J) represent the locations. See also Appendices 7 and 3 for the PFAS concentrations in eggs and soil, respectively.
PFAS: Per- and polyfluoroalkyl substances (the names of the PFAS can be found in Appendix 1).

Appendix 11 Characteristics of the hens and how they were kept at sixty locations, as reported in the source investigation questionnaire.

	% of 60 locations	**Median /Mean (range)**
Mean age of hens (years)	N=0 missing	Median: 2.3 Mean: 2.5 Range: 0.5–6
Chicken breed Laying hen No laying hen Both types Unknown	 12 % 77 % 8.3 % 3.3 %	
How many eggs do your hens lay on average per week? (Mean of all hens)	N=0 missing	Median: 3.4 Mean: 3.6 Range:0.7–7
Have your older hens moulted yet? Yes No Missing	 72 % 28 % 0 %	
Do your hens lay eggs all year round? Yes No Missing	 47 % 53 % 0 %	
Do you provide your hens with supplemental lighting with lamps? Yes No Missing	 5.0 % 95 % 0 %	
Have your hens spent most of the past six months indoors or outdoors? Indoors Outdoors Both Missing	 15 % 67 % 17 % 1.7 %	
Access to worms Yes No Do not know	 73 % 20 % 6.7 %	
Squared meters ground unpaved	N=0 missing	Median: 15 Range:0–60,000
Squared meters ground paved	N=0 missing	Median: 3 Range:0–66

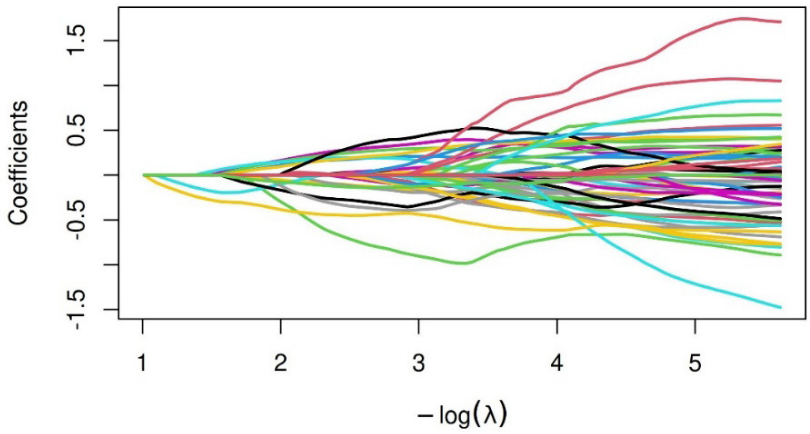
Appendix 12 Supporting graphs from LASSO regression

Figure A2 Selection of the optimal regularisation parameter lambda (λ) in LASSO regression using 10-fold cross-validation.



The plot shows the mean cross-validated mean squared error (MSE) for each value of -log(λ). The left dashed vertical line represents the most regularised model within one standard error of the minimum (lambda.1se) (λ = 0.36 = -log(0.36) = 1.01) while the right dashed vertical line indicates the λ-value that minimises the MSE (lambda.min) (λ = 0.26 = -log(0.26) = 1.3). The optimal λ was chosen on the basis of the smallest MSE. The number of selected variables (nonzero coefficients) for each λ is shown along the upper axis.

Figure A3 LASSO coefficient paths as a function of the regularisation parameter.



Each line represents the estimated value of a regression coefficient as a function of -log(λ), where λ is the regularisation parameter. As -log(λ) increases (moving right along the x-axis), more coefficients get a positive or negative value, illustrating variable selection by LASSO. The plot provides insight into how the model complexity changes with different levels of regularisation. The first variables which are nonzero as the penalty decreases are: Keeping indoors/outdoors in past 6 months: Indoors (yellow); Access to earthworms: No (light blue, below 0); Mean age: 3rd tertile '2.7 to 6 years of age' (light blue, above 0) and Percentage ground unpaved: 2nd tertile '77% to 99.5%' (pink).

Appendix 13 Variables of the source investigation questionnaire, with graphs and information on data handling and model entry

https://www.rivm.nl/bibliotheek/rapporten/2025-0170.xlsx

Appendix 14 Supplementary graphs visualising data distribution and categorisation

The graphs below visualise data distributions and categorisations of the four most predictive variables in the LASSO regression.

Figure A4 Concentrations of summed PFAS in nanogram (ng) per gram in eggs versus the variable whether the hens were indoors and/or outdoors for the last 6 months.

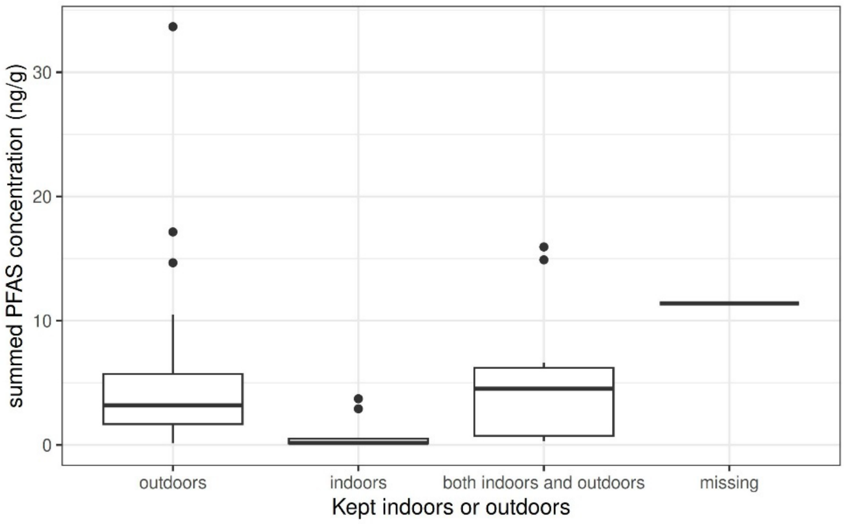


Figure A4 presentsboxplots of the several categories of the variable on the x-axis and the summed PFAS concentration on the y-axis. Categories are: Outdoors n=40 [reference category], indoors n=9, both indoors and outdoors n=10, missing n=1.

Figure A5 Concentrations of summed PFAS in nanogram (ng) per gram in egg versus the variable whether the hens had access to worms.

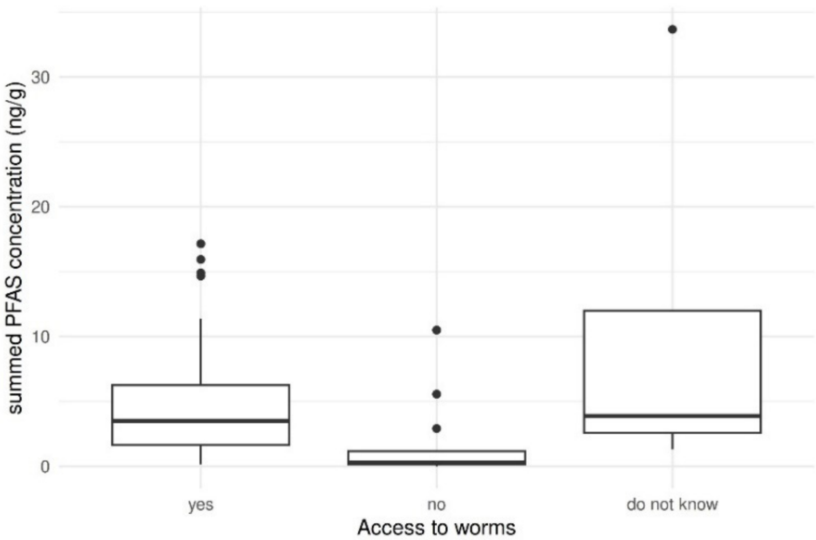


Figure A5 presents boxplots of the several categories of the variable on the x-axis and the summed PFAS concentration on the y-axis. Categories are: Yes n=44 [reference category], no n=12, do not know n=4.

Figure A6 Concentrations of summed PFAS in nanogram (ng) per gram in egg versus the variable mean age of chickens in years.

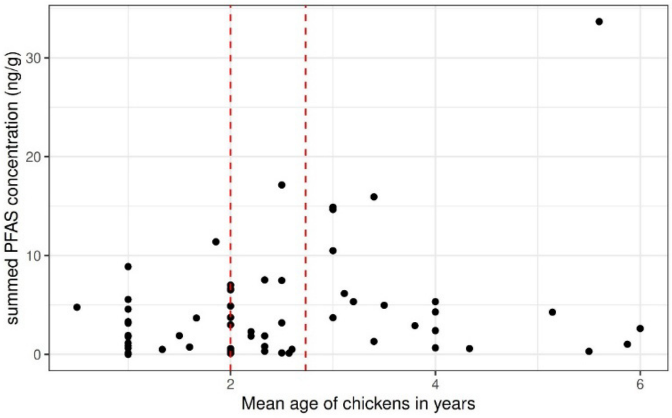


Figure A6 presents a scatterplot with the summed PFAS concentration on the y-axis and the mean age of hens in years on the x-axis. Each point represents an individual observation, red dashed lines are tertile borders and are the categories in the model: T1 (0.5–2.0 years) [reference category], T2 (2.0–2.73 years), and T3 (2.73–6.0 years). Hens aged 2 years fall into T2.

Figure A7 Concentrations of summed PFAS in nanogram (ng) per gram in egg versus the variable percentage unpaved ground.

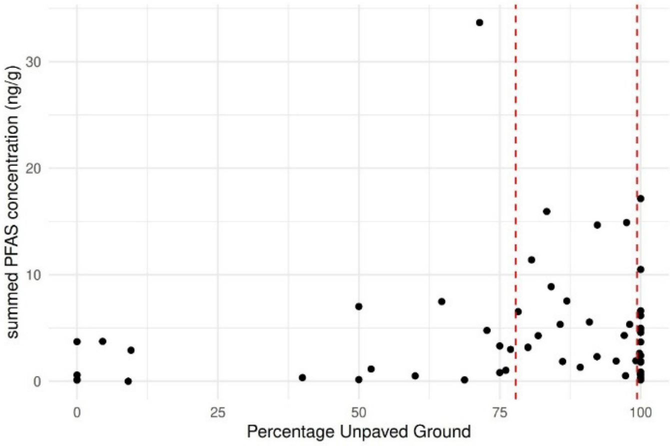
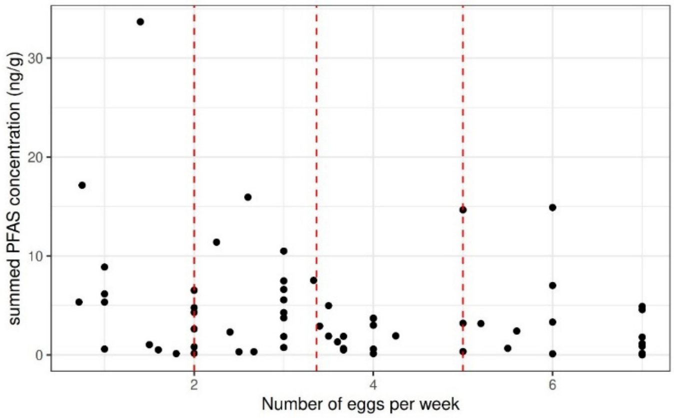


Figure A7 presents a scatterplot with on the y-axis the summed PFAS concentration and on the x-axis the percentage of unpaved ground. Each point represents an individual observation, red dashed lines are tertile borders and are the categories in the model: T1 (<77) [reference category]: T2 (77–99.5): T3 (99.5–100). Missing values were imputed as 0 when chicken owners had answered other questions on uncovered and unpaved ground (interpretation: not filled in because not present). One missing data point was imputed on the basis of the participants' additions at the end of the questionnaire, which provided information on the amount of ground for the hens.

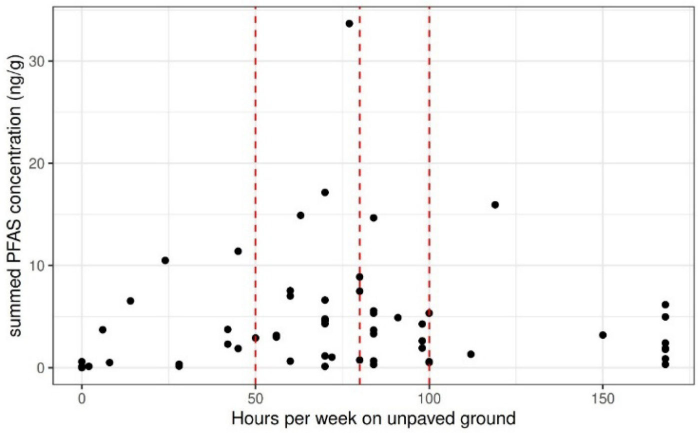
The graphs below visualise data distributions and categorisation for other numerical variables that were entered into the LASSO model as categories, but did not reach the top 4 of predictive variables.

Figure A8 Scatterplot of summed PFAS concentration (ng/g) versus mean egg-laying frequency in number of eggs per week, categorised into quartiles.



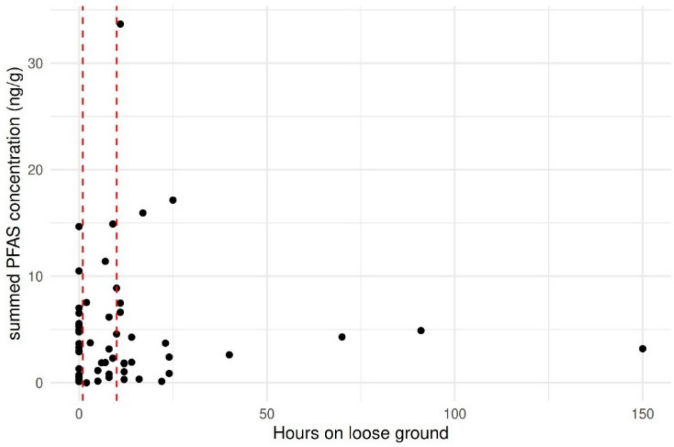
Each point represents an individual observation, red dashed lines are quartile borders: Q1 (0.7–2.0 eggs) [reference category], Q2 (2.0–3.4 eggs), and Q3 (3.4–5 eggs) and Q4: (5–7 eggs). Hens with 2 eggs per week fall into Q1, with 5 eggs per week into Q3.

Figure A9 Scatterplot of summed PFAS concentration (ng/g) versus hours per week on unpaved ground, categorised into quartiles.



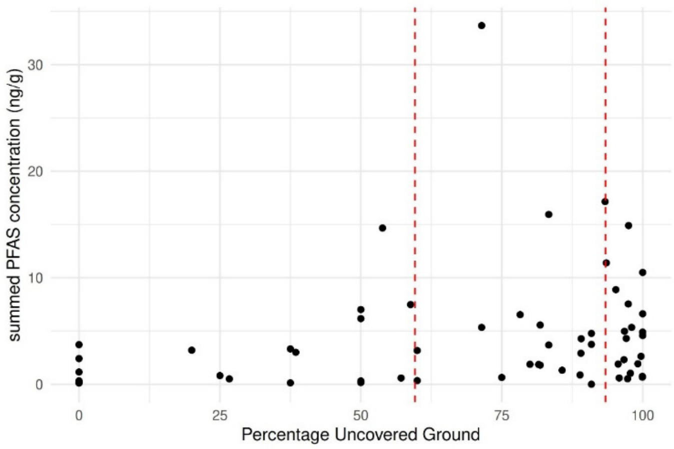
Each point represents an individual observation, red dashed lines are quartile borders: Q1 (0<50) [reference category], Q2 (50–80), and Q3 (80–100) and Q4: (>100). Two locations had missing values: imputed one to zero and one to max amount of hours based on other answers in questionnaire.

Figure A10 Scatterplot of summed PFAS concentration (ng/g) versus hours per week on loose ground, categorised into tertiles.



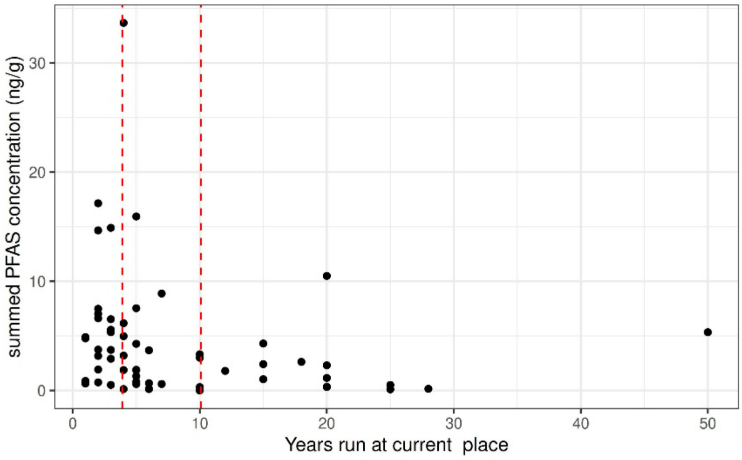
Each point represents an individual observation, red dashed lines are tertile borders: T1 (<1) [reference category]: T2 (1–10): T3 (>10). Missing values were imputed as 0, since the answers to previous questions make clear they have no loose soil..

Figure A11 Scatterplot of summed PFAS concentration (ng/g) versus percentage of uncovered ground, categorised into tertiles.



Each point represents an individual observation, red dashed lines are tertile borders: T1 (<59) [reference category]: T2 (59–93): T3 (>93). Missing values were imputed as 0, when they already have answered other uncovered and unpaved question and one location was imputed on the basis of the description question where the amount of ground was written.

Figure A12 Scatterplot of summed PFAS concentration (ng/g) versus how many years the run of hens is at the current place, categorised into tertiles.



Each point represents an individual observation, red dashed lines are tertile borders: T1 (0–3 years) [reference category]: T2 (4–10 years): T3 (>10 years).

Appendix 15 BSAF earthworm-soil

https://www.rivm.nl/bibliotheek/rapporten/2025-0170.xlsx

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