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FRONT OFFICE FOOD AND PRODUCT SAFETY

Risk assessment of PFASs in feed: Transfer of PFASs detected in feed to edible products of food producing animals and possible animal health risks – Part II: Laying hens and broilers

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Assessment performed by:	RIVM, WFSR and BfR*
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Nederlandse samenvatting

Poly- en perfluoralkyl stoffen (PFAS) zijn een door de mens gemaakte groep chemicaliën die door hun gunstige chemische eigenschappen in veel producten verwerkt zijn of worden. Uitstoot naar het milieu vindt plaats door de productie en het gebruik van deze producten. PFAS worden aangetroffen in grond, grondwater, oppervlaktewater, drinkwater en voedsel, maar ook in biologische matrixen. Een eerdere *Front Office Voedsel- en Productveiligheid* (FO) beoordeling over de overdracht van GenX[1], perfluoroctaanzuur (PFOA) en perfluoroctaansulfonzuur (PFOS) uit slootwater en gras naar voedselproducerende dieren liet mogelijke gezondheidsrisico's zien voor consumenten die voor een lange periode veel zuivelproducten en vlees consumeren van (melk)koeien die uitsluitend worden blootgesteld aan vervuild slootwater en gras (RIVM 2021b). Vanuit het nationaal plan diervoeder zijn PFAS-concentraties in maiskuil, graskuil, luzerne en vismeel onderzocht. Naar aanleiding van deze meetgegevens heeft *Bureau Risicobeoordeling & Onderzoek* (BuRO) aan het FO gevraagd om vast te stellen in welke mate er overdracht is van PFAS in het geanalyseerde voer naar landbouwhuisdieren, of er risico's zijn voor de gezondheid van landbouwhuisdieren na blootstelling aan met PFAS besmet voer, en welke concentraties PFAS in het diervoeder aanwezig mogen zijn voordat maximumgehaltes (ML's) in eetbare producten van landbouwhuisdieren overschreden worden. Recent zijn ML's vastgesteld voor vlees en orgaanvlees van varkens, kippen en runderen. Deze ML's zijn sinds 1 januari 2023 van kracht (EU 2023/915). In deze beoordeling (deel II) worden deze vragen beantwoord voor leghennen en vleeskuikens. In deel I zijn deze vragen beantwoord voor het varken en in deel III voor melkvee en vleesrunderen.

Analyse van de vier diervoeders liet zien dat in graskuil en maiskuil geen PFAS boven de kwantificatielimieten (LOQ's) zijn aangetroffen. Hetzelfde was het geval voor luzerne, met uitzondering van PFOS dat in twee monsters werd aangetroffen. In vismeel zijn veel PFAS gedetecteerd. Graskuil, maiskuil en vismeel wordt niet gevoerd aan volwassen leghennen. Om pikgedrag te voorkomen bij leghennen kan er een kleine hoeveelheid luzerne toegevoegd worden aan hun dagelijkse voer. Aan reguliere vleeskuikens en jonge leghennen wordt geen graskuil, maiskuil en luzerne gevoerd. Wel kan er vismeel gevoerd worden aan vleeskuikens gedurende de eerste week en aan jonge leghennen gedurende

* The German Federal Institute for Risk Assessment (BfR) performed the calculations with the transfer model for eggs

[1] GenX refers to hexafluoropropyleneoxide dimer acid (HFPO-DA), or to its ammonium salt, as used in the GenX technology.

de eerste vier weken van hun leven. Alleen voor de voedermaterialen die gevoederd worden aan leghennen en vleeskuikens is de overdracht berekend. Hierbij is aangenomen dat gehaltes onder de LOQ gelijk zijn aan de LOQ. Dit is een worst-case aanname.

Om de effecten van PFAS op de diergezondheid voor leghennen en vleeskuikens in te schatten is een literatuurstudie uitgevoerd en zijn experts geraadpleegd. Zes artikelen zijn gevonden die aan- of afwezigheid van de gezondheidseffecten van PFAS en/of de kinetiek van PFAS beschrijven en 15 artikelen die de gezondheidseffecten beschrijven na in ovo blootstelling. Er zijn effecten op de gezondheid van kippen vastgesteld, namelijk een verlaging van de concentraties van totaal cholesterol en fosfolipide na een subcutane PFOS-blootstelling. Deze effecten zijn echter gevonden bij een subcutane PFOS blootstelling, die minstens 2600 keer hoger was dan de worst-case orale blootstelling via het geanalyseerde voer. Ook effecten op kuikens na directe injectie van PFAS in het ei werden alleen waargenomen bij concentraties die minimaal 100 maal hoger waren dan de ML voor eieren. In de huidige beoordeling kwamen de geschatte PFAS-concentraties in eieren niet boven de ML. Op basis van deze gegevens zijn geen diergezondheidseffecten te verwachten na blootstelling aan de geanalyseerde concentraties in het voer.

De overdracht van PFOS, PFOA, PFNA en PFHxS naar eieren is geschat met een overdrachtsmodel beschreven door Kowalczyk et al. (2020). De ML's in eieren worden niet overschreden door blootstelling van leghennen via langdurige consumptie van luzerne met de gemeten PFOS gehaltes en met PFOA, PFNA en PFHxS gehaltes gelijk aan of kleiner dan de LOQ's. De ML's in eieren worden ook niet overschreden door blootstelling van leghennen via kortdurende consumptie van vismeel met de gemeten gehaltes van de vier PFAS.

Om de PFAS overdracht naar vlees en organen in jonge leghennen en vleeskuikens kort blootgesteld aan PFAS via vismeel te berekenen, is gebruik gemaakt van een lineair model. Dit leidt ook niet tot een overschrijding van de ML in vlees en orgaanvlees.

Voor de overdracht van PFAS naar vlees en organen (lever en nier) in leghennen die chronisch zijn blootgesteld aan luzerne is een meer verfijnde methode toegepast, namelijk het gebruik van een ei:(orgaan)vlees ratio afgeleid van experimentele data uit de literatuur (Kowalczyk et al. 2020; Feng et al. 2023). Deze ratio's zijn vervolgens vermenigvuldigd met de PFAS concentraties in ei die geschat waren met het overdrachtsmodel voor eieren van Kowalczyk et al. (2020).
De ML's in zowel vlees als orgaanvlees worden niet overschreden voor PFOS, PFOA, PFNA en PFHxS door blootstelling van leghennen via langdurige consumptie van luzerne met gehaltes van deze vier PFAS gelijk aan of kleiner dan de LOQ's.

Daarnaast zijn met behulp van bovenstaande modellen ook de concentraties PFAS in het voer berekend die resulteren in concentraties gelijk aan de ML voor eieren, vlees en orgaanvlees. Voor de leghen en het vleeskuiken waren alle berekende PFAS concentraties in luzerne en vismeel die nodig zijn om de ML's in ei of (orgaan)vlees te bereiken, hoger dan de huidige LOQ.

Voor berekeningen in (orgaan)vlees van leghennen en vleeskuikens kort durig blootgesteld aan het begin van hun leven zijn verschillende (worst-case) aannames gedaan die kunnen leiden tot een overschatting van de daadwerkelijke concentraties in dierlijke producten en daardoor tot een onderschatting van de concentraties in voer die resulteren in vleesgehaltes gelijk aan de ML's. Het verder ontwikkelen van kinetische modellen voor leghennen en het verfijnen van modellen voor vleeskuikens, meer kwantitatieve innamegegevens, het analyseren van relevante voeringrediënten voor vleeskuikens en leghennen en het includeren van precursors van PFAS in de analyse

zullen bijdragen aan een beter inzicht in de daadwerkelijke blootstelling van vleeskuikens en leghennen via diervoeder en resulterende gehaltes in eieren, vlees en orgaanvlees.

Subject

Within the Animal Feed National Plan (NP), various types of animal feed (maize silage, grass silage, lucerne and fishmeal) have been analysed for the presence of PFASs. The Office of Risk Assessment and Research (BuRO) would like to know whether there are risks for animal health following the consumption of PFAS-contaminated feed and maximum levels (MLs) in animal derived products are exceeded when animals are exposed to PFAS-contaminated feed at the reported levels. Recently MLs have been established for meat and offal originating from pig, poultry and bovine animals by the European Commission (EU 2023/915).

Questions

BuRO asked FO the following questions:

1. Is there a risk to animal health when maize silage, grass silage, lucerne and fishmeal contaminated with PFASs (at levels found in the NP 2020) are fed to meat cows, dairy cows, pigs, laying hens and broilers?

2a. What is the transfer of the PFASs (PFOS, PFOA, PFNA, PFHxS) in the above mentioned contaminated feed ingredients to bovine meat/offal, and milk, pig meat/offal, chicken eggs and chicken meat/offal. Compare the estimated concentrations with the MLs of these products.

2b. What is the level of PFASs (PFOS, PFOA, PFNA, PFHxS) in feed (maize silage, grass silage, lucerne and fishmeal) resulting in levels equal to the MLs for PFASs in products of animal origin? In the absence of an ML, please use the current LOQ in that product.

In this assessment, Part II, these questions are answered for laying hens and broilers. In Part I and Part III, the above questions were answered for pigs, and beef cattle and dairy cows. The conclusions in the box below only apply to laying hens and broilers:

Conclusions

1. No risk for the health of laying hens and broilers is expected based on the low PFAS concentrations in feed materials observed in the NP 2020 and the low estimated daily intake of PFASs through these feed materials compared to the PFAS exposure in studies resulting in adverse effects of PFASs.

2a. Grass silage and maize silage are not fed to laying hens and broilers and were therefore not included in this assessment. All four PFASs were detected in fishmeal. Only PFOS was detected in 2 samples of lucerne above the limit of quantification (LOQ). PFOA, PFNA and PFHxS levels in lucerne were below the LOQs.
The egg, meat or offal MLs are not exceeded in laying hens chronically exposed to lucerne and not in laying hens or broilers exposed to fishmeal for a short period at a young age.

2b. Calculated concentrations of the four PFASs in lucerne fed chronically or in fishmeal fed the first four weeks of the life of a laying hen resulting in egg, meat or offal levels equal to the corresponding MLs are above the reported LOQs.

Note: A conservative linear model was used to estimate the transfer of PFAS to meat and offal of broilers and young laying hens exposed to fishmeal. In this model, it is assumed that all PFASs accumulates in either meat or offal, which results in an overestimation of the PFAS concentrations in these edible products (question 2a) and an underestimation of the PFAS concentrations in feed that result in PFAS levels in these edible products equal to the ML (question 2b). To better assess feed to food transfer it is recommended to first develop new, or extend existing, transfer models, including all edible products derived from laying hens and broilers in case other feed ingredients are fed in larger quantities and/or contaminated with higher concentrations of PFAS than the currently analysed lucerne and fishmeal.

1. Introduction

Poly- and perfluoroalkyl substances (PFASs) are a diverse group of man-made chemicals with carbon-fluorine bonds, one of the shortest and strongest bonds known. The fluorinated tail and functional headgroup make PFASs both hydrophobic and hydrophilic, and highly persistent in the environment. As a result of these chemical properties, PFASs are used in many products and industrial processes (e.g. household products, textiles, fire-fighting foam, food packaging materials, construction) and are emitted to the environment through industries and the (re-)use of many PFAS-containing products. Due to these emissions, in combination with the highly persistent nature of PFASs, soil, water and vegetation may be polluted.

PFASs have also been detected in human matrixes, such as blood. For humans, one of the main routes of exposure to PFASs is through the consumption of contaminated food. Food can become contaminated through contaminated soil and water during cultivation of plants, through the accumulation of these substances in animals via feed, water and soil, through PFAS-containing food packaging and/or through PFAS-containing processing equipment. In 2021, the Front Office Food and Product Safety (FO) published the results of a revised risk assessment of GenX and perfluorooctanoic acid (PFOA) in food since the European Food Safety Authority (EFSA) established a tolerable weekly intake (TWI) for the sum of four PFASs (hereafter referred to as EFSA-4; perfluorooctane sulfonic acid (PFOS), PFOA, perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS)) (EFSA 2020; RIVM 2021a).

The transfer of several PFASs in ditch water and silage to edible products of food producing animals was determined in an earlier report (RIVM 2021b). The results of this report showed potential health risks for consumers regularly consuming dairy products and meat from dairy cows solely exposed to contaminated ditch water and grass in a worst-case scenario. Transfer of PFASs to edible products was also seen in laying hens (Kowalczyk et al., 2020).

Within the National Plan Animal Feed in 2020, the presence of PFASs in animal feed (grass silage, maize silage, lucerne and fishmeal) was determined. The results showed that several PFASs were detected in some of the analysed feed materials. As a result, the BuRO asked the FO to determine whether the transfer of PFASs from these feed materials to food products of farm animals (pigs, dairy cows, beef cattle, laying hens and broilers) could result in levels above the maximum levels (MLs), whether there are health risks for farm animals due to intake of these feed materials, and what the levels of PFASs in feed should maximally be before the MLs for PFASs in products of animal origin are exceeded. Recently, MLs have been established for meat and offal originating from pig, poultry and bovine animals by the European Commission (EU 2023/915). In this Part II of the assessment, the questions in relation to laying hens and broilers are addressed.

2. Methods

The methods are described in more detail in Appendix I. Other information used as input and to support the analysis and calculations can be found in Appendices II-V.

a. Analysis of feed samples

In total, 25, 30, 40 and 32 samples of grass silage, maize silage, lucerne and fishmeal, respectively, were analysed according to an internal procedure, SOP-A-1114, at WFSR. Fresh material was extracted using acidified methanol. The final extracts were analysed by

liquid chromatography coupled to tandem mass spectrometry. Isotopically labelled internal standards were added to all samples and quality control samples to allow a more accurate quantification. The complete description of the analysis of the feed samples can be found in Appendix I. The LOQs of the analysis in the four feed materials can be found in Appendix II.

b. Feed consumption data for broilers and laying hens and weight (of food products) during life or at time of slaughter

An overview of the intake of grass silage, maize silage, lucerne and fishmeal for laying hens and broilers is given in Appendix I, Table A1. The intake, the estimated slaughter time and the weight at which food products are produced are based on expert judgement of the department of Animal Nutrition, Wageningen University and Research (Appendix I, Table A2). In short, young hens can occasionally be fed fishmeal during the first 28 days of their life. After this period, laying hens in some cases are only fed a small quantity of lucerne to prevent pecking behaviour. Similarly, just hatched broilers can occasionally be fed fishmeal during the first 7 days of their life. After this period, broilers are not fed any of the analysed feed types.

c. Calculus

i. *Maximum levels of PFASs in eggs, meat and offal*

The transfer of PFASs to food products following exposure to each feed type during each life phase (Question 2a) was calculated with an average and incidental[2] scenario, when available (see Table A1, Appendix I). The PFAS concentrations in eggs, meat and offal were thereafter compared with the MLs (EU 2023/915). In this document the term offal summarises only the liver and kidney. In addition, the estimated concentrations in feed to prevent exceedance of these MLs in food are calculated. In Table 1, the MLs for PFASs (EU 2023/915) are listed. Since only MLs for PFOS, PFOA, PFNA, and PFHxS were established, and BuRO is only interested in these four PFASs, this assessment will not include calculations for other PFASs measured in the feed materials.

Table 1. Maximum levels (MLs) for PFASs in µg/kg wet weight (EU 2023/915).

	PFOS (µg/kg)	**PFOA (µg/kg)**	**PFNA (µg/kg)**	**PFHxS (µg/kg)**	**Sum of 4[a] PFASs**
Eggs	1.0	0.30	0.70	0.30	1.7
Meat of bovine animals, pig and poultry	0.30	0.80	0.20	0.20	1.3
Offal of bovine animals, sheep, pig and poultry	6.0	0.70	0.40	0.50	8.0

[a]: Sum of PFOS, PFOA, PFNA and PFHxS.

ii. *Transfer of PFASs from feed to eggs, meat and offal*

It is unlikely that laying hens and broilers are fed several contaminated feed materials that are only fed sporadically, i.e. fishmeal in young hens and broilers, and lucerne in laying hens. Therefore, in the calculations, the concentrations of PFASs in food products of laying hens and broilers are based on exposure to one single type of feed material during a specific period in their life. Notably, possible precursors of PFOS, PFOA, PFNA and PFHxS were not included in the calculations, since these were not analysed in feed. In addition, it is assumed that the animals have no internal PFASs levels due to previous PFAS exposure (other feed materials, drinking water, soil, or in ovo).

[2] High daily intake that occurs rarely.

For laying hens, a transfer model was available to calculate the transfer from feed to eggs, namely from Kowalczyk et al. (2020). For the transfer from feed to meat and offal no existing model was available so a linear model was used based on conservative consumptions. Only for the transfer from feed to meat and offal in laying hens chronically fed lucerne a more refined approach based on distribution fractions was used, i.e. the egg concentration estimated with the transfer model described by Kowalczyk et al. (2020) linked to the concentration in meat and offal during steady state. The transfer model, linear model and the method based on distribution fractions during steady-state are described in more detail in appendix I and briefly below:

1. Transfer model for eggs

The transfer model developed by Kowalczyk et al. (2020) was used to calculate the concentrations of PFOS, PFOA, PFNA and PFHxS in eggs of laying hens exposed to PFASs through fishmeal. For long-term exposure via lucerne, steady-state transfer rates were used. All calculations using the model were performed by the Bundesinstitut für Risikobewertung (BfR).

a. Use of steady-state assumptions for long-term exposure via lucerne

The concentration in eggs following year round exposure through feed of laying hens can be calculated using steady state assumptions of the laying hen described by Kowalczyk et al. (2020). In this article, the transfer rates from PFASs in feed to food are described for a steady-state situation. Within the experimental exposure period (25 days), steady state is reached for PFOS, PFOA and PFHxS (PFNA is not included in this article). Since this period is considerably short compared to year round exposure, the transfer rates at steady-state were used to calculate the concentrations of PFASs in eggs following exposure of laying hens through lucerne. The transfer rates (unitless) are 0.99 for PFOS and 0.49 for PFOA as calculated by Kowalczyk et al. (2020) and 0.7 for PFHxS as calculated by Wilson et al. (2021). Since the article does not provide a transfer rate for PFNA, this was set at 1.0 based on plausible worst-case assumptions. Feng et al. (2023) described that the gastrointestinal absorption of PFNA was higher than for PFOA and a concentration ratio of matched egg to serum was also higher for PFNA, making it plausible to assume a worst-case assumption for PFNA. Using the following equation, the concentration in the eggs (C_{egg}) at steady state can be calculated:

$$C_{egg} = (TR \cdot I_{Daily\ PFAS}) \,/\, (w_{egg} \cdot LP) \qquad (1)$$

in which TR is the transfer rate, $I_{Daily\ PFAS}$ is the daily intake of PFAS through feed, w_{egg} is the wet weight (ww) of the egg (60 g ww) and *LP* is the laying performance (0.91 eggs/day) (personal communication BfR, based on Kowalczyk et al. (2020)).

b. Use of simulations with time limited exposure via fishmeal

The concentration in eggs following exposure to PFASs for a specific period of time can be simulated using the laying hen model of Kowalczyk et al. (2020) for PFOS, PFOA, PFNA and PFHxS, 60 g ww egg weight, laying performance of 0.91 eggs/day and 17 g ww yolk per egg. Since young hens are exposed when they are not yet laying eggs and this period is not covered by the model, several assumptions were made to calculate the concentrations in eggs at the time of production using the Kowalczyk model. It was assumed that during this period all PFASs are absorbed and no elimination takes place between the intake period and the start of the egg laying phase (worst-case). Once the laying hen has entered its productive egg laying phase, PFAS distribution to ovaria and elimination via eggs starts. In the model this is achieved by administering a single bolus dose at the start of the simulation. During the simulation period this bolus will be cleared

from the system. Therefore, instead of looking at the steady-state PFAS concentration in eggs, here the highest PFAS concentration reached in eggs, in principle at the start of the egg laying period, was compared to the ML.

This method was used to calculate the concentrations of PFOS, PFOA, PFNA and PFHxS in eggs of laying hens exposed through fishmeal.

2. Linear model for meat and offal following time limited exposure via fishmeal

Just hatched broilers and laying hens exposed to fishmeal through feed are slaughtered at a later stage in life. For this model it is assumed that all PFASs will be absorbed and distributed to either meat or offal and no elimination will take place (worst-case assumptions). Due to the anticipated growth of these animals, and since the intake takes place over a relatively short period of time during the early life stages, initial levels will decrease in time. The concentration (C_x) in meat/offal was determined using the following equation:

$$C_x = I_{cum,PFAS} / w_x \qquad (2)$$

in which x stands for meat or offal, $I_{cum,PFAS}$ represents the cumulative intake of PFASs of an animal until slaughter, and w_x represents the weight of the meat or offal (liver plus kidneys) at the moment of slaughter. The intake and the weight of the edible products in relation to the total body weight can be found in Appendix I (Table A1 and A2).

This worst case assumption method was used to calculate the concentrations of PFOA, PFNA, PFHxS and PFOS in meat and offal of broilers and hens exposed to PFASs through fishmeal during an early life stage.

3. Use of distribution fractions for meat and offal following long-term exposure via lucerne

The linear model described above is a pragmatic approach that leads to a certain overestimation of PFAS concentrations in meat and offal. For laying hens chronically fed lucerne, a more refined approach was applied. To this end, experimental distribution data of PFAS (Fang et al. 2023; Kowalczyk et al. 2020) to various tissues were used to derive a ratio between PFAS concentrations in egg and PFAS concentrations in meat (breast) or offal (liver), under the assumption that PFAS levels in eggs, meat and offal are in steady state. The specific egg:meat ratios that were derived were 25.6, 14.9, 28.5 and 10.2 for PFOS, PFOA, PFNA and PFHxS, respectively. The egg:offal ratios were 2.2, 1.4, 2.1 and 2.2 for PFOS, PFOA, PFNA and PFHxS, respectively. For the derived ratios it was assumed that no precursors were present in the feed, eggs or meat; the egg:meat ratio derived from breast meat is representative for all chicken meat and the egg:offal ratio is similar for both liver and kidney. These egg:meat and egg:offal ratios were subsequently used to derive PFAS concentrations in meat and offal, with the following equation

$$C_x = C_{egg} / Ratio_{egg:x} \qquad (3)$$

in which x stands for meat or offal, $Ratio_{egg:x}$ represents the ratio between the PFAS concentration in egg and the PFAS concentrations in meat or offal, at the moment of slaughter (i.e. during steady state). The egg concentrations are derived from the calculated concentrations using equation 1 as described above.

iii. *Concentrations in feed resulting in levels equal to the MLs*

The concentrations of PFOS, PFOA, PFNA and PFHxS in feed based on the MLs for eggs, meat and offal were calculated for the analysed feed materials which can be fed to laying hens and broilers. Possible precursors for these PFASs were not taken into account, since these cannot be measured with the method used to analyse feed materials. These concentrations were also compared to the current LOQs (Appendix II) in feed. The calculations for eggs were done with the transfer model described by Kowalczyk et al. (2020) in case of fishmeal and using TRs for lucerne, in both cases performed by BfR. The calculations for meat and offal were done with a linear model in both laying hens and broilers, except for laying hens chronically fed lucerne where a more refined approach based on distribution fraction was used.

1. Transfer model for eggs

This method was used to calculate the concentrations of PFOS, PFOA, PFNA and PFHxS in lucerne and fishmeal resulting in levels equal to the MLs in eggs of laying hens. All calculations using this model were performed by the BfR.

a. Use of steady-state assumptions for long-term exposure via lucerne

In the case of a feed material continuously fed during the egg-laying period, it is possible to calculate the PFOS, PFOA, PFNA and PFHxS concentrations in feed resulting in levels in eggs of laying hens equal to the MLs by using the steady-state assumptions from Kowalczyk et al. (2020). The PFAS concentration in lucerne resulting in levels equal to the MLs for eggs ($C_{equal,eggs}$) are calculated using the following equations:

$$I_{max,PFAS} = \left(ML_{egg} \cdot w_{egg} \cdot LP\right) / \ TR \qquad (4a)$$

$$C_{equal,egg} = \ I_{max,PFAS} \ / \ (\ I_{daily\ feed}) \qquad (4b)$$

in which $I_{max,PFAS}$ is the maximum intake amount of a certain PFAS, ML_{egg} is the ML for eggs for a certain PFAS, w_{egg} is the wet weight of the egg (60 g ww), *LP* is the laying performance (0.91 eggs/day), *TR* is the transfer rate (modified from Kowalczyk et al. (2020)), and $I_{daily\ feed}$ is the amount of feed material consumed per day. Transfer rates were 0.99 for PFOS, 0.49 for PFOA (Kowalczyk et al. 2020) and 0.7 for PFHxS (Wilson et al. 2021). Since the article did not provide a transfer rate for PFNA, this was set at 1 based on worst-case assumptions.

b. Use of simulations with time-limited exposure via fishmeal

The concentrations in feed resulting in levels in eggs equal to the MLs, following consumption of fishmeal at young age, can be determined using the laying hen model of Kowalczyk et al. (2020), 60 g ww egg weight, 17 g ww yolk weight per egg, laying performance of 0.91 eggs/day. Since young hens are exposed at a time period in which they are not yet laying eggs, additional assumptions in the reverse-calculation were made. The first assumption is that all PFASs are absorbed and no elimination will take place between the intake and the start of the egg laying phase (worst-case). For the model this means one bolus dose equal to the total intake amount over the exposure period is given when the laying hen has entered the egg laying phase. Secondly, distribution to ovaria and elimination of PFASs starts at the time of the bolus dose.

A range of single boluses of the PFAS intakes were simulated by BfR to obtain the single intake amount of PFAS in feed that leads to peak PFAS levels in eggs equal to the corresponding MLs. The obtained PFAS amount was then divided by the total feed intake for both intake scenario's to determine the PFAS concentration that leads to the ML in the respective scenario.

2. Linear model for meat and offal following time-limited exposure via fishmeal

Using the following equations, the concentrations ($C_{equal,x}$) in fishmeal for broilers and laying hens resulting in levels equal to MLs for meat and offal were calculated:

$$I_{max,PFAS} = ML_x \cdot w_x \qquad (5a)$$

$$C_{equal,x} = I_{max,PFAS} / (T \cdot I_{daily\ feed}) \qquad (5b)$$

in which $_x$ stands for meat or offal, $I_{max,PFAS}$ is the maximum intake amount of a certain PFAS during the exposure period, ML_x is the ML of each PFAS in meat or offal (Table 1), w_x is the weight of the meat or offal at the time of slaughter, T is the period in days during which the animal is fed a certain feed type and $I_{daily\ feed}$ is the amount of feed consumption per day (T times $I_{daily\ feed}$ is the exposure scenario found in Table A1 of Appendix I). The weight of the edible products in relation to the total body weight can be found in Appendix I, Table A2. Also for this calculation it is assumed that all PFASs will be absorbed and distributed to either meat or offal, and no elimination will take place (worst-case assumptions).

This linear model is used to calculate the concentrations of PFOA, PFNA, PFHxS and PFOS in fishmeal resulting in levels equal to the MLs in meat/offal of just hatched broilers and young laying hens.

3. Use of distribution fractions for meat and offal following long-term exposure via lucerne

To calculate the PFAS concentrations in lucerne that lead to PFAS concentrations in meat and offal equal to the corresponding MLs, distribution fractions described above, were applied on the PFAS concentrations in eggs estimated with the transfer model.

Specifically, from combining equations 1 and 2, we know that

$$C_{equal,x} = \frac{C_{equal,egg} \cdot ML_x \cdot Ratio_{egg:x}}{ML_{egg}} \qquad (6)$$

where $C_{equal,x}$ is the PFAS concentration in lucerne resulting in levels equal to the MLs for meat or offal and ML_x is the ML for meat or offal. A more elaborate explanation can be found in Annex I

d. Literature search for health effects of PFASs in broilers and laying hens and transfer from feed to food in chickens

A (non-systematic) literature search was carried out to capture relevant literature to determine the health effects of PFASs in laying hens and broilers and relevant transfer parameters/models of PFASs in chickens. The search terms were as follows: 'chemical name' AND (broiler OR hen OR chick* OR poultry) AND (health OR model). In total, six papers describing health effects of chickens (laying hens and/or broilers) exposed to PFASs were identified. In addition, 15 papers describing health effects of hatchlings when exposed in ovo were found. Three papers described kinetic data for laying hens or broilers.

3. Results: PFASs in lucerne and fishmeal

The highest concentrations of each chemical detected per feed material above the LOQ or otherwise the LOQ (< number) fed to laying hens and/or broilers are listed in Table 2. The results of the chemical analysis per sample of the feed materials can be found in Appendices III-IV. The highest concentrations were combined with the maximum feed intakes to calculate the worst-case intake of PFASs. In lucerne, two out of 40 samples showed detectable levels of PFOS with a concentration of 0.068 µg/kg and 0.076 µg/kg. In fishmeal, 30 out of 32 samples showed detectable levels of PFASs. The other two samples were fishmeal made from shrimps. One fishmeal sample contained ten different PFASs, of which five PFASs showed the highest concentrations of all analysed fishmeal samples. The number of samples in which certain PFASs were detected above the LOQ can be found in in brackets in Table 2.

Table 2. Highest concentrations found in the analysed feed samples in µg/kg. When not detected above the limit of quantification (LOQ), the LOQs were listed (in italic, with <). The number of lucerne and fishmeal samples in which a certain PFAS was detected above the LOQ is listed in brackets.

PFAS	***Lucerne (µg/kg)***	***Fishmeal (µg/kg)***
n	*40*	*32*
PFPeA	-	-
PFHxA	*<1.50*	2.70 (1)
PFHpA	*<0.10*	*<0.30*
PFOA	*<0.05*	0.44 (25)
PFNA	*<0.20*	1.50 (26)
PFDA	*<0.20*	1.40 (27)
PFUnDA	*<0.10*	3.10 (27)
PFDoDA	*<0.10*	0.58 (16)
PFTrDA	*<0.10*	2.00 (24)
PFTeDA	*<0.20*	0.29 (14)
PFHxDA	*<0.10*	*<0.02*
PFODA	-	*<1.00*
PFBS	*<0.20*	*<1.00*
PFHxS	*<0.10*	0.55 (7)
PFHpS	*<0.20*	0.18 (6)
PFOS	0.076 (2)	12.00 (19)
PFDS	*<0.20*	*<0.04*
11Cl-PF3OudS	*<0.50*	*<0.20*
9Cl-PF3ONS	*<1.00*	*<0.10*
NaDONA	-	-
GenX	*<1.00*	-

n: number of samples analysed; - : not determined.

4. Results: Transfer of PFASs

The highest measured concentrations or concentrations at the LOQs for feed (in case all concentrations were below LOQ), were combined with the maximum feed intakes using the scenario's from Table A1 (Appendix I) to calculate the highest (incidental[2]) and more realistic (average) PFAS intakes for laying hens and broilers based on the expert judgement of the department of Animal Nutrition, Wageningen Livestock Research. Table

3 shows the estimated concentrations in meat, offal and eggs and whether they exceed the MLs.
Table 4 shows the concentration in each type of feed that would result in concentrations in meat or offal equal to the MLs. To evaluate the current sensitivity of the analytical method, it is also shown whether (and to what extent) these calculated feed concentrations are below the current LOQs. The results are explained in more detail in Appendix I.

a. Laying hens
Laying hens are fed lucerne as a non-feed to prevent pecking behaviour. In addition young hens may be fed fishmeal during the first four weeks of their life. Since PFOA, PFNA and PFHxS were not detected above the LOQ in lucerne, the LOQ was used to calculate the concentrations in eggs, meat and offal of laying hens (worst-case scenario). For PFOS in lucerne and all four PFASs in fishmeal the highest concentrations measured were used for the calculations.

i. *Transfer of PFASs from feed to eggs*
The concentration of PFOS, PFOA, PFNA and PFHxS in eggs was calculated using the steady-state TRs described above (equation 1). Exposure of young hens to fishmeal at a specific time before the egg producing period was simulated with a bolus dose using the model described in Kowalczyk et al. (2020) and the highest concentrations in eggs of that simulation were reported.

The highest steady-state concentrations of PFOS, PFOA, PFNA and PFHxS in eggs of laying hens fed lucerne year round did not exceed the MLs. Also the highest concentration of PFOS, PFOA, PFNA, and PFHxS in eggs of laying hens exposed to fishmeal during their first 28 days of their life, did not exceed the MLs (Table 3).

ii. *Transfer of PFASs from feed to meat and offal*
The concentrations of PFOS, PFOA, PFNA and PFHxS in meat and offal were calculated using equation 2 of the linear model for laying hens exposed to average and incidental[2] fishmeal amounts (see Appendix I, table A 0.1 or 0.5% of fishmeal in the feed) during the first 28 days of their lives or equation 3 based on distribution fraction for laying hens chronically exposed to lucerne (see Table 3 for the results). The steady state concentrations of PFOS, PFOA, PFNA and PFHxS in both meat and offal of laying hens fed lucerne year round did not exceed their MLs. Likewise, feeding of fishmeal to laying hens at the start of their lives for a short period does not lead to exceedance of the MLs for meat or offal for any of the included PFASs, even when no excretion in eggs was assumed.

iii. *Concentrations of PFASs in feed resulting in levels equal to the MLs for eggs*
The concentrations in feed that would result in egg PFAS levels equal to the MLs were calculated by BfR using the TRs and equation 1 for lucerne and the transfer model of an adult laying hen described in Kowalczyk et al. (2020) for fishmeal. For hens exposed to lucerne, equations 4a and b were used to calculate these concentrations based on the steady state. For hens exposed to PFASs during the first four weeks of their lives via fishmeal, two fishmeal concentrations in feed were used as input to obtain the concentration in feed resulting in PFAS levels in eggs equal to the egg MLs.

For all four PFASs the levels in lucerne that would result in levels equal to the ML in eggs are above the reported LOQs. This is also the case for the PFAS levels in fishmeal (Table 4).

iv. *Concentrations PFASs in feed resulting in levels equal to the MLs for meat and offal*
The concentrations in feed that result in meat and offal levels equal to the ML were calculated using equation 5a and 5b of the linear model for laying hens fed fishmeal at a young age and equation 6 for laying hens fed lucerne year-round. Equation 6 is based on distribution fractions. The concentrations of PFOS, PFOA, PFNA and PFHxS in lucerne and fishmeal resulting in levels equal to the MLs for meat and offal in laying hen are all above the reported LOQs in lucerne and fishmeal (Table 4).

b. Broilers

i. *Transfer of PFASs from feed to meat and offal*
Broilers are only exposed to PFASs through fishmeal during a relatively short period at the beginning of their life. Fishmeal is considered a luxury feed type and is not regularly fed to all broilers. The concentrations in meat and offal were calculated using equation 2, assuming that all ingested PFOS, PFOA, PFNA, PFHxS is retained in the body and all PFASs distribute to either the meat or offal. The individual concentrations and the sum of the four PFASs in meat or offal in a broiler at the time of slaughter (38 days) are all below the MLs (Table 3).

ii. *Concentrations in feed resulting in levels equal to the MLs for meat and offal*
The concentrations in fishmeal fed to broilers resulting in levels equal to the MLs for PFOS, PFOA, PFNA, PFHxS were calculated using equation 5a and 5b. All of the calculated concentrations in fishmeal are above the LOQs (Table 4).

Table 3. Overview of the MLs and the concentrations in eggs [a,b], meat [c,d] and offal [c,d] of a laying hen and in meat [d] and offal [d] from a broiler following a short exposure as young hatchling/hen through fishmeal or chronic exposure as an adult laying hen through lucerne. When multiple intake scenarios (average and incidental [e]) are applicable, these are separated by a slash symbol.

	Product	**PFOS (µg/kg)**	**PFOA (µg/kg)**	**PFNA (µg/kg)**	**PFHxS (µg/kg)**	**Sum 4 PFASs [g] (µg/kg)**
MLs	*Eggs*	1.0	0.30	0.70	0.30	1.7
	Meat	0.30	0.80	0.20	0.20	1.3
	Offal	6.0	0.70	0.40	0.50	8.0
Laying hen fed lucerne [f]	*Eggs [a]*	$2.7 \cdot 10^{-3}$	$9.1 \cdot 10^{-4}$	$7.4 \cdot 10^{-3}$	$2.6 \cdot 10^{-3}$	$1.4 \cdot 10^{-2}$
	Meat [c]	$1.1 \cdot 10^{-4}$	$6.0 \cdot 10^{-5}$	$2.6 \cdot 10^{-4}$	$2.6 \cdot 10^{-4}$	$6.9 \cdot 10^{-4}$
	Offal [c]	$1.2 \cdot 10^{-3}$	$6.6 \cdot 10^{-4}$	$3.6 \cdot 10^{-3}$	$1.2 \cdot 10^{-3}$	$6.7 \cdot 10^{-3}$
Laying hen fed fishmeal during first 4 weeks of life	*Eggs [b]*	$3.9 \cdot 10^{-2}$ / 0.20	$6.8 \cdot 10^{-4}$ / $3.4 \cdot 10^{-3}$	$5.9 \cdot 10^{-3}$ / $2.8 \cdot 10^{-2}$	$1.0 \cdot 10^{-3}$ / $4.8 \cdot 10^{-3}$	$4.7 \cdot 10^{-2}$ / 0.24
	Meat [d]	$2.5 \cdot 10^{-2}$ / 0.12	$9.1 \cdot 10^{-4}$ / $4.5 \cdot 10^{-3}$	$3.1 \cdot 10^{-3}$ / $1.5 \cdot 10^{-2}$	$1.1 \cdot 10^{-3}$ / $5.7 \cdot 10^{-3}$	$3.0 \cdot 10^{-2}$ / 0.15
	Offal [c]	0.51 / 2.6	$1.9 \cdot 10^{-2}$ / $9.4 \cdot 10^{-2}$	$6.4 \cdot 10^{-2}$ / 0.32	$2.3 \cdot 10^{-2}$ / 0.12	0.62 / 3.1
Broiler fed fishmeal during first 7 days of life	*Meat [c]*	$1.5 \cdot 10^{-3}$ / $7.3 \cdot 10^{-3}$	$5.3 \cdot 10^{-5}$ / $2.7 \cdot 10^{-4}$	$1.8 \cdot 10^{-4}$ / $9.1 \cdot 10^{-4}$	$6.7 \cdot 10^{-5}$ / $3.3 \cdot 10^{-4}$	$1.8 \cdot 10^{-3}$ /$8.8 \cdot 10^{-3}$
	Offal [c]	$3.0 \cdot 10^{-2}$ / 0.15	$1.1 \cdot 10^{-3}$ / $5.5 \cdot 10^{-3}$	$3.8 \cdot 10^{-3}$ / $1.9 \cdot 10^{-2}$	$1.4 \cdot 10^{-3}$ / $6.9 \cdot 10^{-3}$	$3.6 \cdot 10^{-2}$ / 0.18

[a] : Calculations based on the transfer rates described by Kowalczyk et al (2020) by BfR.

[b] : Calculations based on the transfer model from Kowalczyk et al (2020) by BfR.

[c] : Calculations based on the calculated egg concentrations and distribution fractions, i.e. the egg:meat and egg:offal ratios derived from experimental data described in literature (Fang et al. 2023; Kowalczyk et al. 2020).

[d] : Calculations based on the linear model. Note that due to assumptions, the calculated concentrations are likely to be overestimated.

[e] : High daily intake of fishmeal that occurs rarely.

[f] : Calculations partly based on LOQs, since no PFOA, PFNA and PFHxS were detected above their LOQs in lucerne.

[g] : Sum of PFOS, PFOA, PFNA and PFHxS.

Table 4. Overview of the current feed LOQs and concentrations [a-d] in the feed materials provided to laying hens or broilers resulting in levels equal to the MLs for eggs, meat and offal. When multiple intake scenarios (average and incidental [e]) are applicable, these are separated by a slash symbol.

	Product	***PFOS (µg/kg)***	***PFOA (µg/kg)***	***PFNA (µg/kg)***	***PFHxS (µg/kg)***
Current LOQs	*Lucerne*	$5.0 \cdot 10^{-2}$	$5.0 \cdot 10^{-2}$	*0.20*	*0.10*
	Fishmeal	*1.00*	$2.0 \cdot 10^{-2}$	$2.0 \cdot 10^{-2}$	$5.0 \cdot 10^{-2}$
Laying hen fed lucerne	*Eggs [a]*	27	16	19	12
	Meat [c]	210	670	150	78
	Offal [c]	360	53	22	43
Laying hen fed fishmeal during first 4 weeks of life	*Eggs [b]*	270 / 55	170 / 34	190 / 39	170 / 34
	Meat [d]	150 / 29	390 / 78	97 / 19	97 / 19
	Offal [d]	140 / 28	16 / 3.3	9.4 / 1.9	12 / 2.3
Broiler fed fishmeal during first 7 days of life	*Meat [d]*	$2.5 \cdot 10^3$/ 490	$6.6 \cdot 10^3$ / $1.3 \cdot 10^3$	$1.6 \cdot 10^3$ / 330	$1.6 \cdot 10^3$ / 330
	Offal [d]	$2.4 \cdot 10^3$/ 480	280 / 56	160 / 32	200 / 40

[a] : Calculations based on from the transfer rates described by Kowalczyk et al (2020) by BfR.
[b] : Calculations based on the transfer model from Kowalczyk et al (2020) by BfR.
[c] : Calculations based on the calculated egg concentrations and distribution fractions, i.e. the egg:meat and egg:offal ratios derived from experimental data described in literature (Fang et al. 2023; Kowalczyk et al. 2020).
[d] : Calculations based on the linear model. Note that due to assumptions, the calculations are likely to be underestimated.
[e] : High daily intake of fishmeal that occurs rarely.

5. Question 2a: Transfer of PFASs from feed to edible products from laying hens or broilers

The results of the NP 2020 show that all four PFAS included in this assessment were detected in fishmeal. In lucerne, only PFOS was detected just above the limit of quantification (LOQ) in two samples. Grass silage and maize silage are not fed to laying hens or broilers.

Calculations using the steady-state TRs for lucerne or model described by Kowalczyk et al. (2020) for fishmeal and the measured PFAS concentrations or LOQ levels as exposure concentrations, show that the egg MLs are not exceeded for all four PFASs when the laying hens are chronically fed lucerne or are fed fishmeal for the first four weeks of their lives.
Unfortunately, the transfer model is not suited to calculate PFAS concentrations in meat or offal. Therefore, a linear model was initially employed for these calculations.

Based on the linear model, concentrations of PFOA, PFNA and PFHxS in offal would exceed the corresponding MLs if lucerne with PFAS levels at the LOQ is chronically fed to laying hens (data not shown). Therefore, a more refined approach was taken based on recently published data (Feng et al., 2023; Kowalczyk et al., 2020). This refined approach

is based on the distribution of PFASs between eggs and meat or offal. Based on this refinement, none of the meat or offal MLs were exceeded when lucerne was fed to laying hens.
For fishmeal, none of the meat or offal MLs are exceeded in both laying hens and broilers.

The linear model was still used to calculate PFAS concentration in meat and offal of laying hens and broilers fed fishmeal. However, it has to be noted that these calculated PFAS concentrations are overestimations due to the assumptions made in the linear model used. Although no exceedance of the MLs was seen for feeding fishmeal to laying hens and broilers, more realistic models that cover all four PFASs in all products (egg, meat and offal) in laying hens and broilers are needed, since fishmeal is only fed in small quantities for a short period. A low contamination in feed that is fed in larger quantities may still lead to an estimated exceedance of the MLs. More realistic models could be achieved by extending the existing transfer model for the eggs to more edible products (meat and offal) and adapting the models to include other PFASs as well. Alternatively, generic physiologically-based kinetic models could be used to refine the calculations for more PFASs in edible products in both broilers and laying hens. In addition to the need for these models, the inclusion of other feed materials and lowering of the LOQs of the used analytical methods in feed will also help.

Uncertainties in the PFAS transfer in laying hens and broilers

In this assessment, assumptions for intake and transfer had to be made for PFOS, PFOA, PFNA and PFHxS. First of all, the intake was calculated based on the highest PFAS concentration detected in the feed materials or, in the case of PFOA, PFNA and PFHxS in lucerne, on levels equal to the LOQs as a worst case situation. Based on the actual concentrations in this feed material, the exposure will likely be lower leading to lower transfer to eggs, meat and/or offal. Besides this, the approaches used to calculate transfer have their own uncertainties.

i. *Transfer model for eggs*
The transfer rates described by Kowalczyk et al (2020) were used to calculate the steady state PFAS concentration in eggs. For PFNA no transfer rate was available, so a value of 1 was assumed. This might lead to an overestimation of PFNA concentrations in eggs, e.g. if transfer of PFNA is more similar as that of PFOA. On the other hand, Feng et al. (2023) showed that PFNA is better absorbed than PFOA and that the concentration ratio of egg to serum was also higher for PFNA, making an overestimation less likely.
Uncertainties were also present for the time-limited exposure of laying hens to fishmeal. Time-limited exposure could lead to accumulation in the body followed by a gradual excretion via the eggs once the hens start laying, assuming no other excretion routes from the body are present, except eggs. Because young hens are exposed to fishmeal before they lay eggs and the transfer model described by Kowalczyk et al. (2020) does not cover an exposure period prior to the egg laying phase, several assumptions were made to calculate the concentrations in eggs using the transfer model. As mentioned in the methods section, it was assumed that during this period all PFASs are absorbed and no elimination takes place between the intake period and the start of the egg laying phase (worst-case). This will likely lead to an overestimation of the highest concentration reached in eggs at the start of the productive life of the laying hen. In addition, the model itself could lead to a slight under- or overestimation compared to the actual situation.

ii. *Linear model for meat and offal following time limited exposure via fishmeal*
The linear model used for all PFAS calculations in meat and offal of the laying hen and the broiler, assumes that all PFASs are absorbed and no excretion takes place. However, excretion of PFOS, PFOA and PFHxS, closely related to daily PFAS intake, is seen in eggs and their half-lives are relatively short (Wilson et al. 2021; Kowalczyk et al. 2020; Ehlers, 2012). In addition, Chen et al. (2020) showed that PFOA and PFNA are excreted by broilers. Therefore, the assumption that no excretion takes place, as used for the linear model, leads to an overestimation of the concentrations in meat and offal. Unfortunately, we do not have enough data to quantify how large this overestimation is. Secondly, it was assumed that PFASs do not distribute throughout blood and different organs and tissues in the body, but accumulate solely in either the meat or offal.

Kowalczyk et al. (2020) and Ehlers (2012) showed that only a small part of the applied PFASs is present in the blood, meat, liver and kidneys of laying hens after approximately 25 days of exposure (n=4). They report that from the total intake of PFOS, PFOA and PFHxS, around 3.5% PFOS, 2.3% PFOA and 11% PFHxS was present in blood plasma. Of the ingested amount, around 1.4% PFOS and <1% PFOA and PFHxS was still present in the liver and all three PFASs were <1% present in the kidney. In addition around 2.9% PFOS, 1.0% PFOA and 4.3% PFHxS of the total amount fed was measured in meat. Moreover, Chen et al. (2020) observed that PFOA and PFNA, as metabolites of 8:2 fluorotelomer alcohol administered via oral gavage, were present in the order of magnitude in blood > kidney > liver > meat (muscle). Feng et al. (2023) also observed that PFOA and PFNA were present in the order of magnitude in blood > liver > meat (breast). These data can also be used to estimate the overestimation factor for the assumption that the PFASs solely distribute to either meat or offal. These are a factor 35 for PFOS, a factor 100 for PFOA and a factor 23 for PFHxS in meat. For offal, this will be a 42- to 71–fold overestimation for PFOS and at least a 50-fold overestimation for PFOA and PFHxS. For PFNA similar factors of overestimation could not be estimated due to the lack of existing data.

Yet, one could argue that the concentration of PFASs in the meat of laying hens might be a slight underestimation. The percentage of their bodyweight to retrieve the weight of meat at slaughter of broilers was also used for laying hens. It is likely that laying hens have a lower meat to body weight ratio than broilers, since broilers are kept for their lucrative meat production. For offal this is unclear. As a consequence, PFAS concentrations in meat of laying hens may have been underestimated. Nevertheless, this underestimation is expected to have much lower impact than the overestimations caused by the assumptions related to application of the linear model.

All assumptions described above combined will lead to an overestimation of the actual concentrations of individual PFASs in both meat and offal using the linear model.

iii. *Use of distribution fractions for meat and offal following long-term exposure via lucerne*
During steady state, the distribution fractions were used to calculate to PFAS concentrations in meat and offal. Although more refined than the linear model, this approach also has uncertainties due to the underlying assumptions. These assumptions are that 1) the laying hens are in steady state at the moment of slaughter, 2) the calculated egg concentrations are correct and 3) all types of laying hens have the same distribution fractions. As described in the methods section, the egg:meat and egg:offal ratios were calculated based on experimental PFAS concentrations measured above background in egg, meat and offal (liver). It must be noted that PFOS, PFOA and PFHxS ratios were measured in Lohmann brown laying hens (slaughtered around 31 weeks old),

whereas PFOA and PFNA were measured in Huiyang bearded hens (slaughtered around 24 weeks old). Although these two types of laying hens were relatively young compared to our presumed 77 weeks, a steady-state in egg concentrations for these two species was generally reached. Therefore, the steady-state assumption seems fairly reasonable.

Despite differences in age an breed of laying hens, there was a good agreement between the distribution fractions for PFOA, which was measured in both studies. Nevertheless, it is uncertain whether the distribution fractions are also similar for laying hens that differ greatly in body composition compared to the laying hens used by Kowalczyk et al. (2020), and Feng et al. (2023).

As mentioned in the method section, the precursors of PFOS were not taken into account, because these precursors were not measured in meat and offal by Kowalczyk et al. (2020). However it can be assumed that in steady state, the distribution fraction of a particular PFAS is independent of possible precursors. Besides, in this assessment we also assumed the absence of precursors, since they were not measured. Moreover the laying hens in the experiment described by Feng et al. (2023) were not exposed to precursors.

Another uncertainty related to the use of the distribution fraction is the egg concentration calculated using steady-state assumptions and the transfer rates from Kowalczyk et al. (2020) and Wilson et al. (2020). With the distribution fraction approach, PFAS concentrations in meat and offal are linearly related to the PFAS concentrations estimated for eggs. Uncertainties in the estimated PFAS concentrations in eggs will therefore directly affect the estimation for PFAS concentrations in meat and offal and thus could lead to an under- or overestimation compared to the actual situation.

iv. *Generic uncertainties*
The assumption that laying hens and broilers were only exposed to PFASs through one feed material in their life time and were not exposed in ovo, can lead to an underestimation of the actual concentration of individual PFASs in food products. The actual exposure to PFASs is likely to be higher when exposure to other possible sources such as water, soil, worms/insects and other feed (materials) is taken into account.

Notably, Kowalczyk et al. (2020) showed in their study with laying hens that precursors of some of the PFASs were found in the feed. They suggested that these precursors can be biotransformed in laying hens to PFOS and PFHxS. Chen et al. (2020) showed this for a precursor of PFOA and PFNA in broilers. When determining PFAS transfer into edible products, feed should ideally also be analysed for possible precursors.

6. Question 2b: PFAS concentrations in feed of broilers and laying hens resulting in levels equal to the MLs

The feed concentrations that would result in PFAS levels in eggs, meat and offal equal to the corresponding MLs are shown in Table 4. The calculated concentrations of PFASs in lucerne fed chronically or in fishmeal fed the first four weeks of the life of a laying hen are all above the reported feed LOQs. As described above, based on the assumptions made in the linear model, the calculated concentrations are expected to be lower than the actual concentrations needed to reach levels in edible products that are equal to the MLs. However, since fishmeal is only fed in small quantities, a similar calculation for feed that is fed in larger quantities may lead to a much lower estimated PFAS concentration in feed resulting in PFAS concentrations in eggs, meat and offal that are equal to the ML. To

reduce the underestimation of the calculated concentrations using the linear model, further development of the models is needed, as described in section 5 question 2a.

Uncertainties in concentrations in feed resulting in levels equal to the MLs
The concentrations in feed resulting in levels in egg, meat and offal equal to the MLs are for all PFASs above their LOQs for lucerne and fishmeal. More insight into the feed consumption may reduce the uncertainty in the intake of PFASs.

Similar uncertainties for the different approaches as described for Question 2a are present. These assumptions can lead to an underestimation or overestimation of the concentrations in feed resulting in levels equal to the MLs when using the transfer model for eggs or the distribution fractions and to an overestimation using the linear model. However, due to the assumptions made about the absorption, distribution and excretion and the fact that all calculated PFAS concentrations in feed ingredients resulting in levels equal to the meat ML were above the LOQs, this will not change the current conclusion.

However, the assumption that animals were only exposed through one type of feed in their life time, might have caused an overestimation of the concentrations in feed that would result in levels equal to the MLs, as animals can be exposed through various sources (e.g. other feed materials, water, soil, worm/insects). To take into account co-exposure to PFASs via several feeds/sources, the PFAS concentrations in feed of laying hens and broilers resulting in levels equal to the MLs may need to be lower.

In this assessment, transfer of PFASs from lucerne and fishmeal were estimated for laying hens and broilers. However, these feed ingredients are not consumed at all or not consumed in big quantities by laying hens and broilers. It would be useful to analyse all relevant feed materials that are consumed by laying hens and broilers or compound feed. The feed ingredients fed to the selected animals can be found in Appendix V.

7. Question 1: Health effects of PFASs in broilers and laying hens

The literature search showed that whilst PFAS exposure in animals is frequently discussed, the effects of exposure on the health of food producing animals are seldomly described. Since variability in the kinetics and dynamics of PFASs has been seen between experimental animal species, it is unclear whether findings in animals other than food producing animals are relevant to answer the question of animal health of food producing animals. Many studies describe the kinetics of various PFASs following short-term or bolus exposure for food producing animals. However, to date, there are only a few studies specifically designed to determine the health effects of PFAS exposure for food producing animals.

The following articles described the health effects on chickens following exposure to PFAS:

Yoo et al. (2009) determined the kinetics and tissue disposition of PFOA and PFOS in white leghorn chickens (*Gallus gallus*). Groups of six male chickens were exposed subcutaneously to 0.1 or 0.5 mg/mL PFOA, 0.02 or 0.1 mg/mL PFOS or vehicle (25 µL/h; ~0.06 or 0.3 mg PFOA/day and 0.012 or 0.06 mg PFOS/day) for four weeks resulting in an stable blood concentration of around 30 and 100 ng/mL PFOS, respectively. Afterwards, most chickens were given a depuration period of four additional weeks. Notably, subcutaneous exposure does not reflect exposure through food. No statistically significant changes in body weight, growth rate, brain to body ratio, kidney to body ratio,

histology and most clinical chemistry parameters were seen over the complete time period. Only the total cholesterol and phospholipid concentrations were decreased following exposure to both PFOS concentrations (Yoo et al. 2009).

Yeung et al. (2009) also determined the accumulation and biochemical responses of in chickens exposed to several PFASs. Juvenile chickens (groups of 12) were exposed via oral gavage to mixtures of PFOS, PFOA and PFDA at a low (0.1 mg PFOS/kg bw + 0.1 mg PFDA/kg bw + 0.1 mg PFOA/kg bw) or high (1.0 mg PFOS/kg bw + 1.0 mg PFDA/kg bw + 1.0 mg PFOA/kg bw) dose and vehicle. Chickens were exposed to an initial dose followed by three repeat doses per week for three weeks, resulting in a total of ten doses. Afterwards, part of the chickens were given a depuration period of three additional weeks, whereas the other part was slaughtered to determine accumulation directly following exposure. No treatment-related mortalities and changes in the body weight, liver to body ratio, kidney to body ratio, brain to body ratio, histology and clinical biochemistry parameters were observed following exposure to both mixtures over the complete time period (Yeung et al. 2009).

Tarazona et al. (2015) studied male chickens *(Gallus gallus domesticus* breed "Rubio label *campero")* that were fed 0.2 µg/kg bw PFOS by oral gavage three days a week (equivalent to a daily dose of 0.085 µg/kg bw/day) (3 control and 6 treatment animals). Feeding started at 8 weeks of age and lasted 102 days, followed by 129 days of depuration. No treatment related effects were observed on body weight, mortality or clinical effects.

In Kowalczyk et al. (2020), 12 laying hens (*Lohmann brown*) were exposed to PFAS-contaminated feed for 25 days. Afterwards, 8 hens were given a depuration period of an additional 42 days. Feed was provided *ad libitum*, but intake was recorded. The following intakes of PFAS and PFOS precursors (in µg/hen/day): 0.5 PFBA, 2.5 PFPeA, 1.7 PFHxA, 0.3 PFHpA, 0.6 PFOA, <1.6 (LOQ) PFNA, 4.4 PFBS, 2.1 PFHxS, <1.6 (LOQ) PFHpS, < 0.2 (LOQ) PFAA, 2.8 PFOS, <1 (LOQ) PFDS, 0.6 FOSA, 0.5 FOSAA, 4.8 MeFOSAA and 3.8 EtFOSAA) were determined. No treatment-related health effects were observed during the complete time period of 42 days on body weight, total egg weight and feed intake (Kowalczyk et al. 2020).

Wilson et al. (2021) also determined the effects of PFAS exposure in laying hens (Hy-Line Brown). Hens (22-25 per group) were exposed via drinking water to four PFASs (PFOS, PFHxS, PFOA and PFHxA) with each at the target concentrations of 0.3, 3, 30 and 300 µg/L or at concentrations below the laboratory limit of reporting (vehicle) for 61 days. On a minimum of five occasions each week, hens were examined by veterinarians with additional qualification in poultry health and husbandry. No treatment-related effects on health, welfare or behavioural outcomes were noted over the complete time period. No effects were seen on body weight and egg production. Authors state that 'the lack of any clinical impact of treatment on the health of the birds and on weight gain in the birds during the study can be interpreted as an indication of the lack of observable adverse health effects of PFAS compounds at these levels when adult hens are exposed' (Wilson et al. 2021).

In a biotransformation and tissue bioaccumulation of 8:2 fluorotelomer alcohol study in broilers by Chen et al. (2020) no health effects were observed due to the parent compound or its metabolites among which PFOA and PFNA. In this study male Cobb broilers were intra-gastrically exposed to 5 mg/kg bw 8:2 FTOH for 7 consecutive days. Highest concentrations of PFOA observed were 1094 µg/kg, 2059 µg/kg and 88.5 µg/kg,

in respectively liver, kidney and muscle. For PFNA these concentration were respectively 25 µg/kg, 28 µg/kg and 3.1 µg/kg.

In addition, many studies were found looking at the health of hatchlings when the eggs were injected with PFASs. In these studies, different PFASs were injected directly into the egg.
Pippability has been observed to be reduced when eggs were exposed to 100 mg PFOS/kg egg, but not at 5 mg/kg egg or lower (O'Brien et al., 2009, Briels et al., 2018). Also exposure to 38 mg/kg egg PFHxS reduced pipping success, but 9.3 mg/kg did not (Cassone et al., 2012). PFOS has also been shown to reduce hatchability after exposure to 0.1 mg/kg egg or higher (Molina et al., 2006). However, others found no effect at concentrations until 5 mg/kg egg (Peden-Adams et al., 2009). No effects on pippability and hatchability have been observed for PFOA until concentrations as high as 5 mg/kg egg (Jiang et al. 2016, Ni et al., 2023, Xu et al., 2021).
PFOS significantly reduced embryo survival at exposure levels of 10 mg/kg egg, but not at 3 mg/kg or lower (Nordén et al., 2016, Briels et al., 2018, Strömqvist et al., 2012). Also PFOA increased mortality at concentrations of 1.6 mg/kg egg or higher, but not at 1 mg/kg egg or lower (Nordén et al., 2016, Jiang et al., 2012, Kmecick et al., 2019, Mattson et al., 2015).
Furthermore, in ovo PFOA exposure has been shown to reduce right ventricular wall thickness and heart rate in hatchlings at concentrations of 1 mg/kg egg or higher, but not at concentrations of 0.5 mg/kg egg or lower (Guo et al., 2022, Jiang et al., 2012, Jiang et al., 2016, Ni et al., 2023, Lv et al., 2018). At one and three months of age, instead of wall thinning, an increase in right ventricular wall thickness was observed, accompanied by cardiomyocyte hypertrophy and fibrosis (Ni et al., 2023).
Increased liver weight and steatosis have been observed in hatchlings after in ovo exposure to PFOA (0.5 mg/kg egg or higher) and PFOS (1 mg/kg egg or higher, but not 0.1 mg/kg egg) (Jiang et al., 2012, Moline et al., 2006, Ni et al., 2023, Xu et al., 2021, Peden-Adams et al., 2009).
Since the abovementioned effects after in ovo exposure are only observed at concentrations that are much higher than the MLs for eggs (EU 2023/915; Table 1), no significant health effects after in ovo exposure are thought to occur. The lowest observed adverse effect level (LOAEL) for effects on hatchlings described above were 100 times higher for PFOS, 126,666 times for PFHxS and 1667 times for PFOA when compared to the ML.

In summary no health effects are observed in broilers and laying hens after oral exposure to PFOS and PFOA up to 1.0 mg/kg bw, i.e. $1.0 \cdot 10^{3}$ µg/kg bw during three weeks. Based on the analysed feed materials, and body weight of laying hens and chickens (appendix I, Table A2), the highest total oral exposures for laying hen are $3.5 \cdot 10^{-2}$ µg PFOS/kg bw and $2.3 \cdot 10^{-2}$ µg PFOA/kg bw via lucerne and $7.6 \cdot 10^{-2}$ µg PFOS/kg bw and $2.7 \cdot 10^{-3}$ µg PFOA/kg bw via fishmeal. For broilers the highest total oral exposures are $4.6 \cdot 10^{-3}$ µg PFOS/kg bw and $1.6 \cdot 10^{-4}$ PFOA/kg bw via fishmeal. Thus, the highest PFOS exposure considered in the current assessment is at least $1.3 \cdot 10^{5}$ times lower than the highest oral PFOS exposure described in literature at which no health effects were observed. For the PFOA exposure used in the current assessment this was even at least $4.3 \cdot 10^{5}$ times lower. The only health effect, i.e. a reduction of total cholesterol and phospholipid concentrations was seen after subcutaneous exposure to PFOS resulting in a PFOS concentration in blood around $3.0 \cdot 10^{-2}$ to $1.0 \cdot 10^{-1}$ µg/mL. Based on the analysed feed materials, and the assumptions that the amount of blood is 7.1% of the body weight of the laying hen or broiler (Lautz et al., 2020), all PFOS is absorbed, not excreted and only distributed to blood (worst-case), the highest resulting PFOS blood concentration is $2.9 \cdot 10^{-4}$ µg/mL PFOS via lucerne, $6.3 \cdot 10^{-4}$ µg/mL PFOS via fishmeal for laying hens and $2.7 \cdot 10^{-5}$ µg/mL

PFOS via fishmeal for broilers. Thus, the highest PFOS blood concentration resulting from the current assessment is at least 47 to 3700 times lower than the exposure at which a reduction of total cholesterol and phospholipids was observed in male chickens. It is unlikely that adverse health effects are expected. In addition, the adverse effects observed in hatchlings, after PFASs were directly injected into the egg, were only observed at concentrations >100 fold higher than the ML for eggs. In the present assessment all concentrations found in eggs were well below the ML. Therefore, no negative health effects on hatchlings are to be expected.

8. Recommendations

To reduce the number of assumptions made, and go from worst-case to a more realistic scenario, a better understanding of transfer and exposure is needed in the future:

- The model by Kowaczyk et al. (2020) could be extended to include other tissues, such as muscle and offal. Alternatively, a generic physiologically-based kinetic models could be used to refine the calculations. By extending this model, the model could give quantitative estimates of PFAS levels in meat and offal of laying hens and meat and offal of broilers. Hence, such a model would replace the linear model that describes the worst-case scenario.
- Currently, only a subset of the feeds are considered, resulting in an incomplete picture of PFAS exposure. It would be useful to analyse other feed materials that are consumed in higher quantities by broilers and laying hens and to gain more insight in the feed intake for broilers and laying hens for these and other more relevant feed materials.
- Since exposure to precursors of PFASs, next to PFASs, can affect the total concentration of PFASs in laying hens and broilers, it is recommended to include known precursors in the analysis of the various feed materials.

9. Conclusions and answers

1. Is there a risk to animal health when maize silage, grass silage, lucerne and fishmeal contaminated with PFASs (at levels found in the NP 2020) are fed to laying hens or broilers?

No risk for the health of laying hens and broilers is expected based on the low PFAS concentrations in feed materials observed in the NP 2020 and the low estimated daily intake of PFASs through these feed materials compared to the PFAS exposure in studies resulting in adverse effects of PFASs.

2a. What is the transfer of the PFASs (PFOS, PFOA, PFNA, PFHxS) in the above mentioned contaminated feed ingredients to chicken meat/offal/eggs? Compare the estimated concentrations with the maximum levels (MLs) of these products.

Grass silage and maize silage are not fed to laying hens and broilers and were therefore not included in this assessment. All four PFASs were detected in fishmeal. Only PFOS was detected in 2 samples of lucerne above the limit of quantification (LOQ). PFOA, PFNA and PFHxS levels in lucerne were below the LOQs.
The egg, meat or offal MLs are not exceeded in laying hens chronically exposed to lucerne and not in laying hens or broilers exposed to fishmeal for a short period at a young age.

2b. What is the maximum level of PFASs (PFOS, PFOA, PFNA, PFHxS) allowed in feed materials (maize silage, grass silage, lucerne and fishmeal) before the MLs for PFASs in animal products are exceeded?

Calculated concentrations of the four PFASs in lucerne fed chronically or in fishmeal fed the first four weeks of the life of a laying hen resulting in egg, meat or offal levels equal to the corresponding MLs are above the reported LOQs.

Note: A conservative linear model was used to estimate the transfer of PFAS to meat and offal of broilers and young laying hens exposed to fishmeal. In this model, it is assumed that all PFASs accumulates in either meat or offal, which results in an overestimation of the PFAS concentrations in these edible products (question 2a) and an underestimation of the PFAS concentrations in feed that result in PFAS levels in these edible products equal to the ML (question 2b). To better assess feed to food transfer and risk to animal health, it is recommended to first develop new, or extend existing, transfer models, including all edible products derived from laying hens and broilers in case other feed ingredients are fed in larger quantities and/or contaminated with higher concentrations of PFAS than the currently analysed lucerne and fishmeal.

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Appendix I: Detailed description of methods and results of transfer of PFASs in feed to edible products of broilers and laying hens

1. Methods

a. Analysis of feed samples

The samples were analysed according to an internal procedure SOP-A-1114 at WFSR. One to five grams of fresh material (depending on the product) were extracted using acidified methanol. The extracts were cleaned using weak anion-exchange (WAX) solid phase extraction. After evaporation of the eluate, the residue was dissolved in mobile phase. The final extracts were analysed by liquid chromatography coupled to tandem mass spectrometry. Two ion transitions per compound were monitored according to international guidelines. Isotopically labelled internal standards were added to all samples and quality control samples (including ^{13}C-PFOA and ^{13}C-GenX) to allow a more accurate quantification.

As quality control, a calibration line was prepared in a relevant related product (e.g. silage or fishmeal) with addition of the PFASs from 0 to 5 ng/g. Additionally, chemical blanks were included in duplicate. Furthermore, with every series of samples, a random selection of samples was analysed as is and with addition of a relevant concentration of the PFASs (in some cases additional lower spike levels). Methods used for analysis were validated and accredited under the flexible scope. The limits of quantification (LOQs) can be found in Appendix II.

In total, 25, 30, 40 and 32 samples of grass silage, maize silage, lucerne (chunks, bales, pellets or packs) and fishmeal (fishmeal, salmon meal, pure shrimps, shrimp meal, tuna meal), respectively, have been analysed. The choice to analyse these four animal feeds within the National Plan Animal Feed is based on the conclusion of a report on the risks in the animal feed chain (BuRO 2019). In this report it is concluded that contamination of animal feed plays a role when animals are fed crops (grass, maize) from contaminated locations, and that fishmeal applied in feed can contribute to the exposure to PFASs (BuRO 2019). To answer question 1, 2a and 2b, only the concentrations of PFOS, PFOA, PFNA, PFHxS and the sum thereof were used, since for these PFASs MLs for animal derived products are available (EU 2023/915).

b. Feed consumption of broilers and laying hens

There is hardly any recent published information on the composition of the feed of laying hens and broilers. As a result, the amount, duration and type of feed fed to laying hens and broilers described in this section is estimated based on the expert judgement of the department of Animal Nutrition, Wageningen Livestock Research. Intake is displayed as 88% dry matter (dm). An overview of the intake of lucerne and fishmeal for the laying hens and broilers is given in Table A1.

To prevent pecking behaviour, laying hens are fed an estimated 2 g dm/day lucerne in addition to the normal feed. As a result, laying hens are fed ~730 g dm lucerne on a yearly basis (~2 g dm/day * 365 days/year) during their laying period of 57 weeks. Young hens can occasionally be fed fishmeal during the first four weeks (28 days) of their life. Young hens may be fed max 0.1% (average scenario) to max 0.5% (incidental[2] scenario) of 75 g (= a portion of their daily food intake). Thus, young hens are fed max 2.1 g (0.1% * 75 g/day * 28 days; average scenario) to max 10.5 g (0.5% * 75 g/day * 7 days; incidental[2] scenario) fishmeal (Table A1).

Broilers are not fed grass silage, maize silage or lucerne at any stage of their life. Lucerne is to a limited extent used as diversion material (non-feed). Broilers are not regularly fed fishmeal, but fishmeal is occasionally fed as a starter feed during the first 7

days of their life. As a result, just hatched broilers may be fed max 0.1% (average scenario) to max 0.5% (incidental scenario[2]) of 25 g (= a portion of their daily food intake). Over the 7 days, broilers are fed max 0.175 g (max 0.1% * 25 g/day * 7 days; average scenario) to max 0.875 g (0.5% * 25 g/day * 7 days; incidental[2] scenario) fishmeal (Table A1).

Table A1. Feed consumption of broilers and laying hens when fed the various feed materials

Phase	***Scenario***	***Lucerne[a]***			***Fishmeal***		
		dm/day	*days*	*total[c] (dm)*	*dm/day*	*days*	*total[c] (dm)*
Laying hen	*Average*	~2 g	*57* · 7 = 399	0.798 kg	-	-	-
Young hen (0-28 days)	*Average*	-	-	-	0.1% · 75g	28	2.1 g = $2.1 \cdot 10^{-3}$ kg
	Incidental [b]	-	-	-	0.5% · 75g	28	10.5 g = $1.05 \cdot 10^{-2}$ kg
Just hatched broiler (0-7 days)	*Average [b]*	-	-	-	0.1% · 25g	7	0.175 g = $1.75 \cdot 10^{-4}$ kg
	Incidental [b]	-	-	-	0.5% · 25g	7	0.875 g = $8.75 \cdot 10^{-4}$ kg

[a] : Lucerne is fed as a non-feed to prevent pecking behaviour.
[b] : High daily intake that occurs rarely.
[c] : total = dm/day times days.
- : not fed, dm: dry matter.

c. Calculus (input values)

Table A2. Weight and age at which laying hens and broilers are slaughtered for production.

Animal (phase)	***What type of food (products)?***	***When are these produced?***	***% of weight is meat***	***% of weight is offal (liver + kidneys)***
Laying hen	Eggs + meat + liver + kidney	Eggs at 20 weeks till 77 weeks, Meat/offal at end of production period, at 1.7 kg [a] (77 weeks [c])	60% [d]	2.9% [d]
Broiler	Meat + liver + kidney	Meat/offal at 2.4 kg [a]	60% [b]	2.9% [b]

[a] : The estimated slaughter time and the weight at time of slaughter are based on expert judgement of Paul Bikker (department of Animal Nutrition, Wageningen University and Research). This weight is reached at circa 38 weeks.
[b] : Source: van Raamsdonk et al. (2007): For a broiler of 2.50-2.80 kg: 60.0% meat (muscle) and 2.90% offal (2.10% liver + 0.80% kidney).
[c]: Source: van Raamsdonk et al. (2007).
[d]: Assumed same ratio as broiler chickens.

2. Results: Transfer of PFASs

a. *Laying hen fed lucerne*
Lucerne is fed to laying hens as a non-feed to prevent pecking behaviour. The concentrations detected in lucerne can be found in Table 2 (main text). Since PFOA, PFNA and PFHxS were not detected above the LOQ, the LOQ was used to calculate the concentrations in eggs, meat and offal of laying hens (worst-case scenario).

i. *Transfer of PFOS, PFOA, PFNA and PFHxS from feed to egg*
The concentrations in the eggs following year round exposure were calculated using the transfer rates of Kowalczyk et al. (2020) by the Bundesinstitut für Risikobewertung (BfR). Kowalczyk et al. (2020) describes the transfer rates for PFASs in feed to eggs for a steady-state situation. In approximately 25 days, PFASs are close to/in steady-state in the laying hen. Since this period is short compared to year round exposure, the transfer rates were used to calculate the concentrations of PFASs in eggs following exposure of laying hens through lucerne. The concentration in the eggs (C_{egg}) at steady state can be calculated using equation 1 (main text).

The highest concentrations detected in lucerne can be found in Table 2 (main text). As PFOA, PFNA and PFHxS are not detected above the LOQ, the LOQ was used to calculate the concentrations in eggs of laying hens (worst-case scenario). These concentrations or LOQs (in µg/kg) were multiplied by the daily average intake (0.002 kg/day; Table A1) to obtain the daily intake amount of PFASs ($I_{Daily.\ PFAS}$) (Table A3, a). The $I_{Daily.\ PFAS}$ was subsequently multiplied by the transfer rates (PFOS: 0.99; PFOA: 0.49; PFHxS: 0.7; PFNA: 1) and divided by the weight of an egg (60 g wet weight (ww)) times the laying performance (0.91 egg/day). These transfer rates of PFOS and PFOA were retrieved from (Kowalczyk et al. 2020, whereas the transfer rate of PFHxS was obtained from (Wilson et al. (2021). The transfer rate for PFNA was set at 1 based on plausible worst-case assumptions.
The PFAS concentrations in eggs can be found in Table A3, b. The PFAS concentrations in eggs are all below their respective MLs (Table A3, c).

Table A3. MLs and concentrations of PFAS in eggs of a full grown laying hen [a] following exposure to PFASs through lucerne [b] during their productive phase.

	(a) PFAS intake amount (µg/day)	***(b) Concentration in egg (µg/kg)***	***(c) MLs (µg/kg)***
	Average scenario	*Average scenario*	*Egg*
PFOS	$1.5 \cdot 10^{-4}$	$2.7 \cdot 10^{-3}$	1.0
PFOA	$1.0 \cdot 10^{-4}$	$9.1 \cdot 10^{-4}$	0.30
PFNA	$4.0 \cdot 10^{-4}$	$7.4 \cdot 10^{-3}$	0.70
PFHxS	$2.0 \cdot 10^{-4}$	$2.6 \cdot 10^{-3}$	0.30
Sum 4 PFASs [c]	$8.5 \cdot 10^{-4}$	$1.4 \cdot 10^{-2}$	1.7

[a] : Calculations based on the transfer rates described by from Kowalczyk et al. (2020) by BfR.
[b] : Calculations partly based on LOQs, since no PFOA , PFNA or PFHxS were detected above their LOQs in lucerne.
[c] : Sum of PFOS, PFOA, PFNA and PFHxS.

ii. *Transfer of PFOS, PFOA, PFNA, and PFHxS from feed to meat and offal*
In contrast to the transfer of PFASs to eggs, no transfer model was available to model the transfer of PFASs to meat or offal. However, assuming that PFAS concentrations in laying hen eggs, meat and offal are in steady-state after long-term exposure to lucerne, the concentration in meat and offal can be calculated using equation 3. The calculations were made under the assumptions that 1) the laying hens are in steady state at the moment of slaughter, 2) the calculated egg concentrations are correct and 3) all types of laying hens have the same distribution fractions.

As described above in Appendix I (section 2.a.i.) the highest PFOS concentration detected in lucerne can be found in Table 2 (main text), as well as the LOQ for PFOA, PFNA and PFHxS in lucerne. Since PFOA, PFNA and PFHxS are not detected above the LOQ, the LOQ was used to calculate the concentrations in meat and offal of laying hens (worst-case scenario). These concentrations or LOQs (in µg/kg) were used to calculate the eventual egg concentrations as described above in section 2.a.i. (Appendix I). The resulting egg concentration as shown in Table A3 were subsequently divided by the ratio egg:meat or ratio egg:liver to calculate the concentration in meat and offal (Table A4,a and b) . The egg:meat ratios that were derived from experimental data described in literature (Fang et al. 2023; Kowalczyk et al. 2020) were 25.6, 14.9, 28.5, and 10.2 for PFOS, PFOA, PFNA and PFHxS, respectively. The egg:offal ratios were 2.2, 1.4, 2.1 and 1.0 for PFOS, PFOA, PFNA and PFHxS. (Fang et al. 2023; Kowalczyk et al. 2020)

The results show that exposure of laying hens does not lead to exceedance of the MLs in meat and offal (Table A4, a, b,c).

Table A4. MLs and concentrations of PFASs in meat and offal of a full grown laying hen at the time of slaughter [a] following exposure to PFASs through lucerne [b] during their productive phase.

	(a) Concentration in meat (µg/kg)	***(b) Concentration in offal (µg/kg)***	***(c) MLs (µg/kg)***	
	Average scenario	*Average scenario*	*Meat*	*Offal*
PFOS	$1.1 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$	0.30	6.0
PFOA	$6.0 \cdot 10^{-5}$	$6.6 \cdot 10^{-4}$	0.80	0.70
PFNA	$2.6 \cdot 10^{-4}$	$3.6 \cdot 10^{-3}$	0.20	0.40
PFHxS	$2.6 \cdot 10^{-4}$	$1.2 \cdot 10^{-3}$	0.20	0.50
Sum 4 PFASs [c]	$6.9 \cdot 10^{-4}$	$6.7 \cdot 10^{-3}$	1.3	8.0

[a] : Calculations based on the calculated egg concentrations (see table A3) and distribution fractions, i.e. the egg:meat and egg:offal ratios derived from experimental data described in literature (Fang et al. 2023; Kowalczyk et al. 2020).
[b] : Calculations partly based on LOQs, since no PFOA, PFNA and PFHxS were detected above their LOQs in lucerne.
[c] : Sum of PFOS, PFOA, PFNA and PFHxS.

iii. *Concentration in lucerne resulting in levels equal to the MLs for eggs*
It is possible to calculate the PFOA, PFNA, PFHxS and PFOS-concentrations in feed resulting in levels in eggs of laying hens equal to the proposed MLs by using the steady-

state assumptions of the laying hen model of Kowalczyk et al. (2020). These concentrations were calculated by BfR using equation 3 (main text).

The daily intake amount ($I_{max,PFAS}$) of laying hens during year round daily exposure to lucerne (Table A5, a) was calculated by multiplying the MLs for the corresponding PFAS for egg (Table 1, main text) with the weight of an egg ($6.0 \cdot 10^{-2}$ kg) multiplied by LP ($=6.0 \cdot 10^{-2}$ kg/egg*0.9 egg/day = 0.054 kg/day) and subsequently dividing it by the PFAS transfer rates (PFOS: 0.99; PFOA: 0.49; PFHxS: 0.7; PFNA:1) (Kowalczyk et al. 2020; Wilson et al. (2021). The transfer rate for PFNA was set at 1 based on plausible worst-case assumptions. Next, the maximal daily intake of each PFAS was divided by the maximal daily feed intake of the average intake scenario's (0.002 kg/day; Table A1) to obtain the concentration in lucerne resulting in levels equal to the MLs for PFASs in eggs (Table A5, b).

None of the concentrations in lucerne resulting in levels equal to the egg ML are below the LOQ.

Table A5. Current LOQs and concentrations of PFASs in lucerne fed to a laying hen resulting in levels equal to the MLs for eggs of a laying hen during production [a].

	(a) Max. daily intake amount (µg/day) resulting in egg ML	***(b) Concentration in lucerne (µg/kg) resulting in egg ML***	***(c) Current LOQs in lucerne (µg/kg)***
PFOS	$5.5 \cdot 10^{-2}$	27	$5.0 \cdot 10^{-2}$
PFOA	$3.3 \cdot 10^{-2}$	16	$5.0 \cdot 10^{-2}$
PFNA	$3.8 \cdot 10^{-2}$	19	0.20
PFHxS	$2.3 \cdot 10^{-2}$	12	0.10

[a]: Calculations based on the transfer transfer rates described by Kowalczyk et al. (2020) by BfR.

iv. *Concentration in lucerne resulting in levels equal to the MLs for meat and offal*
The concentrations in lucerne that would lead to levels equal to the MLs for meat or offal of adult laying hens were calculated using the distribution fractions, i.e. equation 6 (main text).

This equation was derived as follows. First, by combining equations 1 and 3 (main text), we know that:

$$C_x = \frac{(TR \cdot I_{Daily\ PFAS}) \,/\, (w_{egg} \cdot LP)}{Ratio_{egg:x}} \qquad \text{(A1)}$$

in which C_x is the PFAS concentration in tissue x (meat or offal), TR is the transfer rate, $I_{Daily\ PFAS}$ is the daily intake of PFAS through feed, w_{egg} is the wet weight (ww) of the egg (60 g ww) and LP is the laying performance (0.91 eggs/day).

Since we are interested in the amount of daily PFAS intake that leads to a C_x that is equal to the corresponding ML ($I_{max,x}$), equation A1 can be rewritten into:

$$I_{max,x} = \frac{ML_x \cdot Ratio_{egg:x} \cdot w_{egg} \cdot LP}{TR} \qquad \text{(A2)}$$

Where ML_x represent the ML of tissue x. Combining A2 with equation 4a (main text) gives

$$\frac{I_{max,x}}{I_{max,egg}} = \frac{ML_x \cdot Ratio_{egg:x}}{ML_{egg}} \qquad \text{(A3)}$$

Substituting equation 4b (main text) into equation A3 finally gives equation 6 presented in the main text

Since $C_{max,egg}$ is calculated using the transfer model, and the MLs and the distribution ratios are known, it is possible to calculate a value for $C_{max,x}$, the PFAS concentration in lucerne that leads to a C_x that is equal to the corresponding ML.

None of the concentrations in lucerne resulting in PFAS concentrations that match the ML for meat or offal are below the LOQ.

Table A6. Current LOQs and concentrations of PFASs in lucerne fed to a laying hen resulting in levels equal to the MLs for meat or offal of a laying hen at the time of slaughter[a].

	(a) Max. daily intake amount (µg) resulting in ML for		***(b) Concentration in lucerne (µg/kg) resulting in ML for***		***(c) Current LOQs in lucerne (µg/kg)***
Product	*Meat*	*Offal*	*Meat*	*Offal*	
PFOS	0.42	0.72	210	360	$5.0 \cdot 10^{-2}$
PFOA	1.30	0.11	670	53	$5.0 \cdot 10^{-2}$
PFNA	0.31	$4.5 \cdot 10^{-2}$	150	22	0.20
PFHxS	0.16	$8.5 \cdot 10^{-2}$	78	43	0.10

[a] : Calculations based on the calculated egg concentrations and distribution fractions, i.e. the egg:meat and egg:offal ratios derived from experimental data described in literature (Fang et al. 2023; Kowalczyk et al. 2020).

b. Young laying hen fed fishmeal

Young laying hens can be exposed to PFASs through fishmeal during the first 28 days of their life. Young hens may be fed a total of max $2.1 \cdot 10^{-3}$ kg (average scenario) to max $1.05 \cdot 10^{-2}$ kg (incidental[2] scenario) fishmeal (Table A1). The concentrations detected in fishmeal can be found in Table 2 (main text).

i. *Transfer of PFASs from fishmeal to eggs*

The maximum concentrations in eggs following exposure through fishmeal fed during the first 28 days of the life of young laying hens was calculated by BfR using the model of an adult laying hen described by Kowalczyk et al. (2020). Since the hens are exposed for 4 weeks, roughly 16 weeks before the production of eggs starts and the model is based on an adult laying hen producing eggs the following assumptions were made. All PFASs are absorbed and no elimination will take place between the intake and the start of the egg laying phase (worst-case).To simulate this, the distribution to ovaria and elimination of PFASs starts at the time of the bolus dose. Note that for the model one bolus dose equal to the cumulative intake amount (Table A7, a) over the 28-day exposure period is taken into account. The cumulative PFAS intake during 28 days (Table A7, a) is calculated by multiplying the highest concentration measured in feed (in µg/kg; Table 2, main text) by the cumulative intake (average total intake: $2.1 \cdot 10^{-3}$ kg; incidental[2] total intake: $1.05 \cdot 10^{-2}$ kg; Table A1).

To run the simulation, the weight of the egg was set at 60 g ww, the egg yolk at 17 g, the laying performance at 0.91 eggs/day.The total intake amount (Table A7,a) was used as input for the model and the model provided the highest reached concentration in eggs for each scenario for PFOA, PFOS and PFHxS.

The highest concentration in eggs following the total intake during the first 28 days of the life of a laying hen can be found in Table A7, b. None of the highest concentrations found in eggs are above the MLs for PFAS in eggs. The concentrations in eggs decline quickly since no additional exposure takes place to fishmeal during the adult life of the laying hen.

Table A7. MLs and concentrations of PFAS in eggs of a full grown laying hen [a] following exposure to PFASs through fishmeal during the first 28 days of their life.

	(a) Cumulative PFAS intake amount in 28 days (µg)		***(b) Concentration in egg (µg/kg)***		***(c) MLs (µg/kg)***
	Average scenario	*Incidental scenario [b]*	*Average scenario*	*Incidental scenario [b]*	*Eggs*
PFOS	$2.5 \cdot 10^{-2}$	0.13	$3.9 \cdot 10^{-2}$	0.20	1.0
PFOA	$9.2 \cdot 10^{-4}$	$4.6 \cdot 10^{-3}$	$6.8 \cdot 10^{-4}$	$3.4 \cdot 10^{-3}$	0.30
PFNA	$3.4 \cdot 10^{-3}$	$1.6 \cdot 10^{-2}$-	$5.9 \cdot 10^{-3}$	$2.8 \cdot 10^{-2}$	0.70
PFHxS	$1.2 \cdot 10^{-3}$	$5.8 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$	$4.8 \cdot 10^{-3}$	0.30
Sum 4 PFASs [c]	$3.1 \cdot 10^{-2}$	0.16	$4.7 \cdot 10^{-2}$	0.24	1.7

[a]: Calculations based on the transfer model from Kowalczyk et al. (2020) by BfR.
[b]: High daily intake that occurs rarely.
[c]: Sum of PFOS, PFOA, PFNA and PFHxS.

ii. *Transfer of PFASs from fishmeal to meat and offal*

The concentrations in meat and offal of the laying hen at the time of slaughter through fishmeal exposure were calculated using equation 2 (main text).

The highest PFAS concentrations detected in fishmeal can be found in Table 2 (main text). The total PFAS intake ($I_{cum.\ PFAS}$) (Table A8, a) is calculated by multiplying the concentration in fishmeal (in µg/kg) by the cumulative intake (average intake: $2.1 \cdot 10^{-3}$ kg; incidental[2] intake: $1.05 \cdot 10^{-2}$ kg; Table A1). To calculate the concentration of PFASs in meat (Table A8, b) and offal (Table A8, c) at the time of slaughter, the total PFAS intake ($I_{cum.\ PFAS}$) for both intake scenarios was divided by the amount of meat (60% of 1.7 kg= 1.02 kg) or offal (kidneys + liver, 2.9% of 1.7 kg= 0.0493 kg) of a fully grown laying hen at the time of slaughter (Table A2). This calculation was made under the worst-case assumption that all PFASs are absorbed, no elimination will take place (including eggs) and that all PFASs will go to either meat (b) or offal (c). For this calculation it is also assumed that a laying hen has the same proportions of meat and offal as a broiler. As a consequence, PFAS concentrations in meat and offal of laying hens may have been underestimated. Nevertheless, this underestimation is expected to have much lower impact than the overestimations caused by the assumptions related to application of the linear model.

The concentrations of PFOS, PFOA, PFNA, PFHxS, and the sum thereof in meat and offal are all below the MLs (Table A8, d).

Table A8. MLs and concentrations of PFAS in meat and offal of a full grown laying hen at the time of slaughter [a] following exposure to PFAS through fishmeal during their newly hatched phase.

	(a) Cumulative PFAS intake amount (µg)		***(b) Concentration in meat (µg/kg)***		***(c) Concentration in offal (µg/kg)***		***(d) MLs (µg/kg)***	
	Average scenario	*Incidental scenario [b]*	*Average scenario*	*Incidental scenario [b]*	*Average scenario*	*Incidental scenario [b]*	*Meat*	*Offal*
PFOS	$2.5 \cdot 10^{-2}$	0.13	$2.5 \cdot 10^{-2}$	0.12	0.51	2.6	0.30	6.0
PFOA	$9.2 \cdot 10^{-4}$	$4.6 \cdot 10^{-3}$	$9.1 \cdot 10^{-4}$	$4.5 \cdot 10^{-3}$	$1.9 \cdot 10^{-2}$	$9.4 \cdot 10^{-2}$	0.80	0.70
PFNA	$3.2 \cdot 10^{-3}$	$1.6 \cdot 10^{-2}$	$3.1 \cdot 10^{-3}$	$1.5 \cdot 10^{-2}$	$6.4 \cdot 10^{-2}$	0.32	0.20	0.40
PFHxS	$1.2 \cdot 10^{-3}$	$5.8 \cdot 10^{-3}$	$1.1 \cdot 10^{-3}$	$5.7 \cdot 10^{-3}$	$2.3 \cdot 10^{-2}$	0.12	0.20	0.50
Sum 4 PFASs [c]	$3.0 \cdot 10^{-2}$	0.16	$3.0 \cdot 10^{-2}$	0.15	0.62	3.1	1.3	8.0

[a] : Calculations based on the linear model. Note that due to assumptions, the calculated concentrations are likely to be overestimated.
[b] : High daily intake that occurs rarely.
[c] : Sum of PFOS, PFOA, PFNA and PFHxS.

iii. *Concentration in feed resulting in levels equal to the MLs for eggs*

The concentrations of PFOS, PFOA, PFNA and PFHxS in feed resulting in levels in eggs equal to the MLs were estimated manually by BfR using the laying hen model of Kowalczyk et al. (2020), 60 g ww egg weight, laying performance of 0.91 eggs/day and the transfer rates (unitless) described above (0.99 for PFOS; 0.49 for PFOA; 0.7 for PFHxS (Kowalczyk et al. 2020; Wilson et al. 2021) and 1.0 for PFNA (read across, plausible worst-case). Since young hens are exposed at a time period in which they are not yet laying eggs, several assumptions in the reverse-calculation were made. The first is that all PFASs are absorbed and no elimination will take place between the intake and the start of the egg laying phase (worst-case). For the model this is simulated as if one bolus dose equal to the total intake amount over the exposure period is given when the laying hen has entered the egg laying phase. Secondly, distribution to ovaria and elimination of PFASs starts at the time of the bolus dose.
A range of single boluses of the PFAS intakes were simulated by BfR to estimate the maximum intake amount of PFAS in feed present in single bolus resulting in peak levels equal to the MLs of eggs (Table A9, a). These obtained maximal intakes of PFASs were divided by the maximal fishmeal intake of both intake scenario's (average intake: $2.1 \cdot 10^{-3}$ kg; incidental[2] intake: $1.05 \cdot 10^{-2}$ kg; Table A1) to obtain the concentration in feed resulting in levels equal to the MLs for PFAS in eggs of laying hens (Table A9, b).

None of the concentrations in fishmeal fed during the first 28 days of the laying hens life resulting in levels equal to the egg ML are below the LOQs.

Table A9. Current LOQs and concentrations of PFASs in fishmeal fed to a laying hen during the first 28 days of life resulting in levels equal to the MLs for eggs of a laying hen during production [a].

	(a) Max. intake amount (µg) resulting in egg ML	***(b) Concentration in fishmeal (µg/kg) resulting in egg ML***		***(c) Current LOQs in fishmeal (µg/kg)***
Scenario		*Average*	*Incidental [b]*	
PFOS	0.58	270	55	1.00
PFOA	0.36	170	34	$2.0 \cdot 10^{-2}$
PFNA	0.36	190	39	$2.0 \cdot 10^{-2}$
PFHxS	0.41	170	34	$5.0 \cdot 10^{-2}$

[a] : Calculations based on the model from Kowalczyk et al. (2020) using manual optimalisation.
[b] : High daily intake that occurs rarely.

iv. *Concentration in feed resulting in levels equal to the MLs for meat and offal*
In contrast to the transfer of PFASs to eggs, no transfer model was available to model the transfer of PFOS, PFOA, PFNA and PFHxS to meat or offal. Therefore a linear model was used where all ingested PFASs end up in either meat or in offal. For the calculations equation 4 (main text) was used.

The $I_{max,PFAS}$ for PFAS from fishmeal fed to laying hens for 28 days (Table A10, a) was calculated by multiplying the MLs for the corresponding PFAS for meat and offal (Table 1, main text) with the amount of meat (60% of 1.7 kg= 1.02 kg) or offal (kidneys + liver, 2.9% of 1.7 kg= 0.0493 kg) of a laying hen at the time of slaughter (Table A2). Next, these maximal intakes of PFASs when exposed at the ML for meat and offal were divided by the maximal intake of both intake scenario's (average intake: $2.1 \cdot 10^{-3}$ kg; incidental[2] intake: $1.05 \cdot 10^{-2}$ kg; Table A1) to obtain the concentration in fishmeal resulting in levels equal to the MLs for PFASs in meat of laying hens (Table A10, b). This was repeated for the MLs of PFASs in offal of laying hens (Table A10, b). The PFAS concentrations in feed leading to levels in meat and offal equal to the MLs would increase with a decrease in intake amount. Since the maximal intake of the scenarios was used, the estimated PFAS concentrations are likely an underestimation of the actual concentrations needed to exceed the MLs in meat and offal. For this calculation it was assumed that all PFASs are absorbed, no elimination of PFASs took place between intake and slaughter, all PFASs accumulate in either meat or offal. In addition it was also assumed that a laying hen has the same proportions of meat and offal as a broiler chicken, which could overestimate the total PFAS intake in broiler to reach the ML in meat compared to actual laying hens. The latter might lead to a slight overestimation of the concentration in feed resulting in levels equal to the MLs. However, this potential overestimation is much lower than the underestimation due to the worst-case assumptions.

All PFAS concentrations in fishmeal that would result in levels equal to either ML are below the LOQ.

Table A10. Current LOQs and concentrations of PFASs in fishmeal fed to laying hens during the first 4 weeks resulting in levels equal to the MLs for meat or offal at the time of slaughter [a].

	(a) Max. intake amount (µg) resulting in ML for		***(b) Concentration in fishmeal (µg/kg) resulting in ML for***				***(c) Current LOQs in fishmeal (µg/kg)***
Product	*Meat*	*Offal*	*Meat*		*Offal*		
Scenario	*Both[b]*	*Both[b]*	*Average*	*Incidental[b]*	*Average*	*Incidental[b]*	
PFOS	0.31	0.30	150	29	140	28	1.00
PFOA	0.82	$3.5 \cdot 10^{-2}$	390	78	16	3.3	$2.0 \cdot 10^{-2}$
PFNA	0.20	$2.0 \cdot 10^{-2}$	97	19	9.4	1.9	$2.0 \cdot 10^{-2}$
PFHxS	0.20	$2.5 \cdot 10^{-2}$	97	19	12	2.3	$5.0 \cdot 10^{-2}$

[a] : Calculations based on the linear model. Note that due to assumptions, the calculations are likely to be underestimated.
[b] : High daily intake that occurs rarely.

c. Broilers fed fishmeal

Broilers are not fed grass silage, maize silage or lucerne but may be exposed to PFASs through fishmeal during the first week of life.

i. *Transfer of PFASs from feed to meat and offal*

The concentration in the edible products of broilers at the time of slaughter, exposed through fishmeal as just hatched broilers, were calculated using equation 2 (main text).

The highest concentrations detected in fishmeal can be found in Table 2 (main text). These concentrations (in µg/kg) were multiplied by the cumulative fishmeal intake for the two scenario's (average intake: 1.75×10^{-4} kg; incidental[2] intake: 8.75×10^{-4} kg; Table A1) to obtain the cumulative PFAS intake ($I_{cum.\ PFAS}$) (Table A11, a). The cumulative intake ($I_{cum.\ PFAS}$) of the two scenarios was divided by the amount of meat (60% of 2.4 kg= 1.44 kg) or offal (kidneys + liver, 2.9% of 2.4 kg= 0.07 kg) of a broiler at the time of slaughter (Table A2) to calculate the concentration in meat and offal (Table A11, b and c). This calculation was made under the worst-case assumption that all PFASs are absorbed, no elimination will take place and that all PFASs will go into either the meat (b) or the offal (c). Hence, the concentrations in meat or offal are likely to be overestimated by the linear model used.

The results show that none of the concentrations predicted in meat or offal following exposure of just hatched broilers to fishmeal leads to exceedance of the MLs of meat and offal.

Table A11. MLs and concentrations of PFASs in meat and offal of a full grown broiler at the time of slaughter [1] following exposure to PFASs through feed containing fishmeal during their newly hatched phase.

	(a) Cumulative PFAS intake amount (µg)		***(b) Concentration in meat (µg/kg)***		***(c) Concentration in offal (µg/kg)***		***(d) MLs (µg/kg)***	
	Average scenario	*Incidental scenario [b]*	*Average scenario*	*Incidental scenario [b]*	*Average scenario*	*Incidental scenario [b]*	*Meat*	*Offal*
PFOS	$2.1 \cdot 10^{-3}$	$1.1 \cdot 10^{-2}$	$1.5 \cdot 10^{-3}$	$7.3 \cdot 10^{-3}$	$3.0 \cdot 10^{-2}$	0.15	0.30	6.0
PFOA	$7.7 \cdot 10^{-5}$	$3.8 \cdot 10^{-4}$	$5.3 \cdot 10^{-5}$	$2.7 \cdot 10^{-4}$	$1.1 \cdot 10^{-3}$	$5.5 \cdot 10^{-3}$	0.80	0.70
PFNA	$2.6 \cdot 10^{-4}$	$1.3 \cdot 10^{-3}$	$1.8 \cdot 10^{-4}$	$9.1 \cdot 10^{-4}$	$3.8 \cdot 10^{-3}$	$1.9 \cdot 10^{-2}$	0.20	0.40
PFHxS	$9.6 \cdot 10^{-5}$	$4.8 \cdot 10^{-4}$	$6.7 \cdot 10^{-5}$	$3.3 \cdot 10^{-4}$	$1.4 \cdot 10^{-3}$	$6.9 \cdot 10^{-3}$	0.20	0.50
Sum 4 PFASs [c]	$2.5 \cdot 10^{-3}$	$1.3 \cdot 10^{-2}$	$1.8 \cdot 10^{-3}$	$8.8 \cdot 10^{-3}$	$3.6 \cdot 10^{-2}$	0.18	1.3	8.0

[a] : Calculations based on the linear model. Note that due to assumptions, the calculated concentrations are likely to be overestimated.
[b] : High daily intake that occurs rarely.
[c] : Sum of PFOS, PFOA, PFNA and PFHxS.

ii. *Concentration in feed resulting in levels equal to the MLs*

The concentrations in fishmeal that would lead to levels equal to the MLs for meat or offal were calculated using the linear model, i.e. equation 4 (main text).

The maximum intake amount ($I_{max,PFAS}$) of broilers during the 7 days period was calculated by multiplying the MLs for the corresponding PFAS for meat and offal (Table 1, main text) by the amount of meat (60% of 2.4 kg= 1.44 kg) or offal (kidneys + liver, 2.9% of 2.4 kg= 0.07 kg) of a broiler at the time of slaughter (Appendix I, Table A2). Next, these intakes of PFASs leading to ML levels for meat and offal were divided by the cumulative feed intake ($I_{cum,feed}$) of the two intake scenario's (average intake: 1.75×10^{-4} kg; incidental[2] intake: 8.75×10^{-4} kg; Table A1) to obtain the concentration in feed resulting in levels equal to the MLs for PFASs in meat of broilers (Table A12, b). This was repeated for the MLs of PFASs in offal of broilers (Table A12, b). Note that these calculations were made under the worst-case assumption that all PFASs are absorbed, no elimination will take place and that all PFASs will go to either meat or offal. Hence, the concentrations in feed resulting in levels equal to the MLs are likely to be higher than the model predictions.

None of the concentrations in fishmeal resulting in levels equal to either ML are below the LOQ.

Table A12. Current LOQs and concentrations of PFASs in fishmeal fed to a broiler resulting in levels equal to the MLs for meat or offal at the time of slaughter [a]

	(a) Max. intake amount (µg/day) resulting in ML		***(b) Concentration in fishmeal (µg/kg) resulting in ML***				***(c) Current LOQs in fishmeal (µg/kg)***
Product	Meat	Offal	Meat		Offal		
Scenario	Both	Both	*Average*	*Incidental*[b]	*Average*	*Incidental*[b]	
PFOS	0.43	0.42	$2.5 \cdot 10^{3}$	490	$2.4 \cdot 10^{3}$	480	1.00
PFOA	1.20	$4.9 \cdot 10^{-2}$	$6.6 \cdot 10^{3}$	$1.3 \cdot 10^{3}$	280	56	$2.0 \cdot 10^{-2}$
PFNA	0.29	$2.8 \cdot 10^{-2}$	$1.6 \cdot 10^{3}$	330	160	32	$2.0 \cdot 10^{-2}$
PFHxS	0.29	$3.5 \cdot 10^{-2}$	$1.6 \cdot 10^{3}$	330	200	40	$5.0 \cdot 10^{-2}$

[a] : Calculations based on the linear model. Note that due to assumptions, the calculations are likely to be underestimated.
[b] : High daily intake that occurs rarely.

References Appendix I

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Appendix II – LOQs for PFAS analysis in µg/kg in feed fed to laying hens and broilers

Full name	***Abbreviation***	***Lucerne***	***Fishmeal***
Perfluoropentanoic acid	PFPeA (C5)	-	-
Perfluorohexanoic acid	PFHxA (C6)	1.50	1.20
Perfluoroheptanoic acid	PFHpA (C7)	0.10	0.30
Perfluoroctanoic acid	PFOA (C8)	0.05	0.02
Perfluornonanoic acid	PFNA (C9)	0.20	0.02
Perfluordecanoic acid	PFDA (C10)	0.20	0.02
Perfluorundecanoic acid	PFUnDA (C11)	0.10	0.04
Perfluordodecanoic acid	PFDoDA (C12)	0.10	0.06
Perfluortridecanoic acid	PFTrDA (C13)	0.10	0.04
Perfluortetradecanoic acid	PFTeDA (C14)	0.20	0.03
Perfluorhexadecanoic acid	PFHxDA (C16)	0.10	0.02
Perfluoroctadecanoic acid	PFODA (C18)	-	1.00
Perfluorbutane sulfonic acid	PFBS (C4)	0.20	1.00
Perfluorhexane sulfonic acid	PFHxS (C6)	0.10	0.05
Perfluorheptane sulfonic acid	PFHpS (C7)	0.20	0.06
Perfluoroctane sulfonic acid	PFOS (C8)	0.05	1.00
Perfluordecane sulfonic acid	PFDS (C10)	0.20	0.04
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	0.50	0.20
9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	9Cl-PF3ONS	1.00	0.10
Sodium dodecafluoro-3H-4, 8 dioxanonanoate	NaDONA	-	-
Hexafluoropropylene oxide dimer acid	GenX/HFPO-DA	1.00	-

- : not determined.

Appendix III – Analytical results of PFASs in lucerne[3]

Number	1	2	3	4	5	6	7	8	9	10
SAMPLE_ID	200598296	200598297	200598298	200598299	200598300	200598718	200599402	200599403	200599404	200599405
VWA CODE	75090498	75090536	75090471	75090528	75090501	75421745	75180829	75180802	75180772	75180799
PRODUCT:	lucerne brok	timothee brok	lucerne brok	esparcette brok	lucerne pakken	plantaardig voedermiddel eu=lucerne	lucernepellets	lucernepellets	lucernepellets	lucernepellets
LAND VAN HERKOMST:	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL
DATUM MONSTERNAME:	18-08-2020	18-08-2020	18-08-2020	18-08-2020	18-08-2020 20	20-08-2020	26-08-2020	26-08-2020	26-08-2020	26-08-2020
PFPeA (ng/g)										
PFHxA (ng/g)	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
PFHpA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFOA (ng/g)	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
PFNA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFDA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFUnDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFDoDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFTrDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFTeDA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFHxDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFODA (ng/g)										
PFBS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFHxS (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFHpS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFOS (ng/g)	<0.050	<0.050	<0.050	0.068	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
PFDS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
11Cl-PF3OUdS (ng/g)	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
9Cl-PF3ONS (ng/g)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
NaDONA (ng/g)										
GenX (ng/g)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

[3] The concentration PFAS is expressed in ng/g. This is equal to µg/kg. In the main text and appendix I and II µg/kg was used as unit for consistency in the units during calculations.

Number	11	12	13	14	15	16	17	18	19	20
SAMPLE_ID	2005994006	2005994007	2005994008	2005994605	2005994606	2005994607	2005994609	2005994710	2006001119	2006001120
VWA CODE	75180764	75421753	75421761	75421877	75421893	75421915	75421907	75421842	75410697	75410719
PRODUCT:	lucernepellets	plantaardig voedermiddel eu= lucerne	plantaardig voedermiddel eu= lucerne	plantaardig voedermiddel eu= lucerne	plantaardig voedermiddel eu= lucerne	plantaardig voedermiddel eu= lucerne	plantaardig voedermiddel eu= lucerne	plantaardig voedermiddel eu= lucerne	lucerne	lucerne
LAND VAN HERKOMST:	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL
DATUM MONSTERNAME:	26-08-2020	26-08-2020	26-08-2020	27-08-2020	27-08-2020	27-08-2020	27-08-2020	27-08-2020	03-09-2020	03-09-2020
PFPeA (ng/g)										
PFHxA (ng/g)	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
PFHpA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFOA (ng/g)	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
PFNA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFDA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFUnDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFDoDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFTrDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFTeDA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFHxDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFODA (ng/g)										
PFBS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFHxS (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFHpS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFOS (ng/g)	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
PFDS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
11Cl-PF3OUdS (ng/g)	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
9Cl-PF3ONS (ng/g)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
NaDONA (ng/g)										
GenX (ng/g)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0				

Number	21	22	23	24	25	26	27	28	29	30
SAMPLE_ID	200600121	200600122	200600123	200600124	200604092	200604094	200604123	200604124	200604126	200604462
VWA CODE	75410689	75180837	75410727	75410743	75410921	75410883	75410913	75410891	75410905	75179197
PRODUCT:	lucerne	lucerne	lucerne	lucernepellets	lucernepellets	lucernepellets	lucernepellets	lucernepellets	lucernepellets	plantaardig voedermiddel eu= lucerne
LAND VAN HERKOMST:	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL
DATUM MONSTERNAME:	03-09-2020	03-09-2020	03-09-2020	03-09-2020	15-10-2020	15-10-2020	15-10-2020	15-10-2020	15-10-2020	20-10-2020
PFPeA (ng/g)										
PFHxA (ng/g)	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
PFHpA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFOA (ng/g)	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
PFNA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFDA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFUnDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFDoDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFTrDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFTeDA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFHxDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFODA (ng/g)										
PFBS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFHxS (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFHpS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFOS (ng/g)	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
PFDS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
11Cl-PF3OUdS (ng/g)	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
9Cl-PF3ONS (ng/g)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
NaDONA (ng/g)										
GenX (ng/g)										

Number	31	32	33	34	35	36	37	38	39	40
SAMPLE_ID	200604830	200604831	200604832	200605761	200605764	200605766	200610448	200610449	200610450	200610455
VWA CODE	75411154	75411162	75411189	75090951	75090935	75090919	75419899	75419902	75419872	75419864
PRODUCT:	lucerne	lucerne	lucerne	lucerne balen	timotee brok	lucerne brok	plantaardig voedermiddel eu= lucerne	plantaardig voedermiddel eu= lucerne	plantaardig voedermiddel eu= lucerne	plantaardig voedermiddel eu= lucerne brok
LAND VAN HERKOMST:	NL	NL	NL	NL	NL	NL	FR	NL	NL	NL
DATUM MONSTERNAME:	27-10-2020	27-10-2020	27-10-2020	03-11-2020	03-11-2020	03-11-2020	03-11-2020	03-12-2020	03-12-2020	03-12-2020
PFPeA (ng/g)										
PFHxA (ng/g)	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
PFHpA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFOA (ng/g)	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
PFNA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFDA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFUnDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFDoDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFTrDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFTeDA (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFHxDA (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFODA (ng/g)										
PFBS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFHxS (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PFHpS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
PFOS (ng/g)	<0.050	<0.050	<0.050	<0.050	0.076	<0.050	<0.050	<0.050	<0.050	<0.050
PFDS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
11Cl-PF3OUdS (ng/g)	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
9Cl-PF3ONS (ng/g)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
NaDONA (ng/g)										
GenX (ng/g)					<1.0					

Appendix IV – Analytical results of PFASs in fishmeal[3]

Number	1	2	3	4	5	6	7	8	9	10
SAMPLE_ID	200578884	200580520	200580521	200582336	200587859	200587860	200590948	200593209	200593333	200596980
VWA CODE	86426226	86026872	86026899	86266997	86198509	86198487	86124637	86210851	86287897	86098547
PRODUCT:	vismeel	vismeel	vismeel	vismeel	vismeel	vismeel	vismeel	vismeel	vismeel	vismeel
LAND VAN HERKOMST:	PE	NL	NL	DE	PE	MA	NL	DE	DE	NL
DATUM MONSTERNAME:	07-01-20	20-02-20	20-02-20	09-03-20	11-05-20	07-05-20	08-06-20	02-07-20	02-07-20	21-07-20
PFPeA (ng/g)										
PFHxA (ng/g)	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	2.7	<1.2	<1.2	<1.2
PFHpA (ng/g)	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
PFOA (ng/g)	0.057	<0.020	0.14	0.066	0.080	0.11	0.088	0.20	0.18	0.068
PFNA (ng/g)	0.058	0.81	0.79	0.23	0.14	0.15	<0.020	0.68	0.57	0.10
PFDA (ng/g)	0.039	0.45	1.0	0.28	0.041	0.10	<0.020	0.66	0.68	0.13
PFUnDA (ng/g)	0.11	0.90	1.8	0.49	0.080	0.20	<0.040	1.6	1.7	0.42
PFDoDA (ng/g)	<0.060	0.24	0.49	0.11	<0.060	<0.060	<0.060	0.46	0.52	0.068
PFTrDA (ng/g)	0.063	0.67	1.3	0.29	<0.040	0.11	<0.040	0.57	0.95	0.17
PFTeDA (ng/g)	<0.030	0.14	0.25	0.064	<0.030	<0.030	<0.030	0.20	0.20	0.075
PFHxDA (ng/g)	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020
PFODA (ng/g)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
PFBS (ng/g)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	
PFHxS (ng/g)	<0.050	0.099	<0.050	0.10	0.083	0.070	<0.050	<0.050	<0.050	<0.050
PFHpS (ng/g)	<0.060	<0.060	0.076	<0.060	x	x	<0.060	0.066	0.078	<0.060
PFOS (ng/g)	<1.0	2.8	7.3	1.9	<1.0	<1.0	<1.0	5.9	6.0	<1.0
PFDS (ng/g)	<0.040	<0.040	<0.040	<0.040	<0.040	<0.040	<0.040	<0.040	<0.040	<0.040
11Cl-PF3OUdS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
9Cl-PF3ONS (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
NaDONA (ng/g)										
GenX (ng/g)										

x: detected

Number	11	12	13	14	15	16	17	18	19	20
SAMPLE_ID	200596987	200596989	200596998	200597076	200597078	200597081	200597082	200597300	200599759	200599954
VWA CODE	86089726	86089661	86550393	86592223	86592258	86426617	86089718	86426625	86287935	86550407
PRODUCT:	vismeel batchnummer: l:b5/20-07-03 tht-datum 03-07-2021 seelowe bioceval cuxhaven de 03 352 0001 08	vismeel cpsp90 handelsdocument 9709 15-05-2020 1000 kg	fischmehl/ fishmeal zak 25 kg vismeel batchnr. 200094 - a8507	voedermiddel vismeel 65% re batch 200140. zakgoedle verdatum 28-07-2020	voedermiddel vismeel 65% re zakgoed tht 17/8/2020	salmonmeal	garnalenmeel batchnummer 15-01-2020productiedatum: 12-01-2020productnummer: 43010300	pure shrimp	vismeel gehydrolis eerd	garnalenmehl batchnummer 17.03.2020
LAND VAN HERKOMST:	-	-	DK	MA	PE	NL	NO	NL	FR	DE
DATUM MONSTERNAME:	22-07-20	22-07-20	27-07-20	29-07-20	29-07-20	25-07-20	22-07-20	25-07-20	31-08-20	31-08-20
PFPeA (ng/g)										
PFHxA (ng/g)	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2			<1.2	
PFHpA (ng/g)	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<7.0	<7.0	<0.30	<7.0
PFOA (ng/g)	0.22	<0.020	0.067	0.11	0.12	0.088	<1.5	<1.5	<0.020	<1.5
PFNA (ng/g)	0.67	1.1	0.12	0.12	0.28	<0.020	<1.0	<1.0	0.73	<1.0
PFDA (ng/g)	0.56	0.92	0.11	0.092	0.17	<0.020	<1.0	1.4	0.60	1.4
PFUnDA (ng/g)	1.2	3.1	0.39	0.20	0.46	<0.040	<1.0	1.5	1.5	1.4
PFDoDA (ng/g)	0.27	0.58	0.077	<0.060	0.12	<0.060	<1.0	<1.0	0.38	<1.0
PFTrDA (ng/g)	0.77	2.0	0.17	0.071	0.28	<0.040			1.5	
PFTeDA (ng/g)	0.16	0.29	0.068	<0.030	0.080	<0.030	<1.0	<1.0	0.17	<1.0
PFHxDA (ng/g)	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.50	<0.50	<0.020	<0.50
PFODA (ng/g)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0			<1.0	
PFBS (ng/g)										
PFHxS (ng/g)	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<2.5	<2.5	<0.050	<2.5
PFHpS (ng/g)	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060			<0.060	
PFOS (ng/g)	3.3	3.3	<1.0	<1.0	2.0	<1.0	<2.0	5.2	3.4	4.9
PFDS (ng/g)	<0.040	<0.040	<0.040	<0.040	<0.040	<0.040	<3.0	<3.0	<0.040	<3.0
11Cl-PF3OUdS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20			<0.20	
9Cl-PF3ONS (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10			<0.10	
NaDONA (ng/g)							<2.0	<2.0		<2.0
GenX (ng/g)										

Number	21	22	23	24	25	26	27	28	29	30
SAMPLE_ID	200599955	200599993	200600924	200601493	200601505	200601507	200601509	200604197	200604821	200604822
VWA CODE	86550423	86550415	86111934	86552566	86287978	86112043	86112078	86529432	86548526	86548534
PRODUCT:	vismeel	zalmmeel	vismeel (marokko fishmehl 65% behandelt)	vismeel (fishbone meal)	zalmmeel	tonijnmeel	garnalenmeel	vismeel batchnr: I:b5/20.03.03productiedatum: 03-03-2020	vismeel. 2202. export.	vismeel. 31287.
LAND VAN HERKOMST:	NO	NO	MA	NL	NL	IT	IT	DE	-	DK
DATUM MONSTERNAME:	31-08-20	31-08-20	08-09-20	14-09-20	14-09-20	14-09-20	14-09-20	12-10-20	23-10-20	23-10-20
PFPeA (ng/g)										
PFHxA (ng/g)	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2		<1.2	<1.2	<1.2
PFHpA (ng/g)	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<7.0	<0.30	<0.30	<0.30
PFOA (ng/g)	0.44	0.13	0.15	0.060	0.039	0.16	<1.5	0.20	0.15	0.20
PFNA (ng/g)	1.5	0.055	0.21	0.26	0.16	0.25	<1.0	0.55	0.27	0.52
PFDA (ng/g)	0.75	<0.020	0.11	0.21	0.14	0.33	<1.0	0.38	0.15	0.34
PFUnDA (ng/g)	1.1	<0.040	0.28	0.43	0.35	1.2	<1.0	1.2	0.55	0.84
PFDoDA (ng/g)	0.24	<0.060	<0.060	0.10	<0.060	0.27	<1.0	0.19	<0.060	0.12
PFTrDA (ng/g)	0.32	<0.040	0.095	0.24	0.20	0.40		0.44	0.36	0.27
PFTeDA (ng/g)	0.068	<0.030	<0.030	0.055	<0.030	0.11	<1.0	<0.030	<0.030	<0.030
PFHxDA (ng/g)	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.50	<0.020	<0.020	<0.020
PFODA (ng/g)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0		<1.0	<1.0	<1.0
PFBS (ng/g)	<1.0							<1.0	<1.0	<1.0
PFHxS (ng/g)	0.55	<0.050	<0.050	0.065	<0.050	<0.050	<2.5	<0.050	<0.050	0.34
PFHpS (ng/g)	0.18	<0.060	<0.060	<0.060	<0.060	<0.060		<0.060	<0.060	0.14
PFOS (ng/g)	12	<1.0	1.0	1.4	<1.0	1.5	<2.0	2.8	2.0	9.8
PFDS (ng/g)	<0.040	<0.040	<0.040	<0.040	<0.040	<0.040	<3.0	<0.040	<0.040	<0.040
11Cl-PF3OUdS (ng/g)	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20		<0.20	<0.20	<0.20
9Cl-PF3ONS (ng/g)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10		<0.10	<0.10	<0.10
NaDONA (ng/g)							<2.0			
GenX (ng/g)										

Number	31	32
SAMPLE_ID	200606480	20060668 7
VWA CODE	86529645	86553104
PRODUCT:	marokkaan s vismeel	vismeel
LAND VAN HERKOMST:	MA	-
DATUM MONSTERNAME:	11-11-20	11-11-20
PFPeA (ng/g)		
PFHxA (ng/g)	<1.2	<1.2
PFHpA (ng/g)	<0.30	<0.30
PFOA (ng/g)	0.19	0.14
PFNA (ng/g)	0.22	0.13
PFDA (ng/g)	0.11	0.11
PFUnDA (ng/g)	0.29	0.29
PFDoDA (ng/g)	<0.060	<0.060
PFTrDA (ng/g)	0.10	0.10
PFTeDA (ng/g)	<0.030	<0.030
PFHxDA (ng/g)	<0.020	<0.020
PFODA (ng/g)	<1.0	<1.0
PFBS (ng/g)	<1.0	<1.0
PFHxS (ng/g)	<0.050	<0.050
PFHpS (ng/g)	<0.060	<0.060
PFOS (ng/g)	1.3	<1.0
PFDS (ng/g)	<0.040	<0.040
11Cl-PF3OUdS (ng/g)	<0.20	<0.20
9Cl-PF3ONS (ng/g)	<0.10	<0.10
NaDONA (ng/g)		
GenX (ng/g)	31	32

Appendix V – Feed regularly fed to laying hens (in Dutch)

Feed composition, for laying hens and broilers . Qualitative, global by descending proportion

Leghennen (legperiode)	**Leghennen (opfokperiode)**	**Vleeskuikens**
Mengvoer	**Mengvoer**	**Mengvoer**
Maïs	Maïs	Tarwe
Tarwe	Tarwe	Mais
Sojaschroot/-schilfers	Zonnebloemschroot/-schilfers	Sojaschroot en -schillen/hullen
Zonnebloemschroot/-schilfers	Sojaschroot/-schilfers	Zonnebloemzaadschroot
Krijt/kalksteen	Sojabonen	Erwten
Gerst	Haver	Haver
Palmolie	Krijt/kalksteen	Sojaolie
Sojaolie	Tarweproducten	Dierlijk vet
Haver	Sojaolie	
Erwten	Kool-, raapzaadschroot/schilfers	

Provided by the department of Animal Nutrition, Wageningen Livestock Research. Based on the availability of the products and the prices in 2020.