National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

WAGENINGEN UNIVERSITY & RESEARCH

FRONT OFFICE FOOD AND PRODUCT SAFETY

RISK ASSESSMENT OF GenX AND PFOA IN FOOD PART 2: TRANSFER OF GenX AND PFOA IN DITCHWATER AND SILAGE TO EDIBLE PRODUCTS OF FOOD PRODUCING ANIMALS

Risk assessment requested by:	Office for Risk Assessment and Research
Risk assessment performed by:	RIVM and RIKILT
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Subject
In the past, the companies DuPont/Chemours in Dordrecht and Custom Powders in Helmond emitted the substances GenX[1] and PFOA into the air and surface water. The emission of GenX by DuPont/Chemours is ongoing. Consequently, the area in the vicinity of these companies (soil, water and vegetation) became polluted, as communicated by the National Institute for Public Health and the Environment (RIVM) with the the city council of Dordrecht in July and September 2018. One of the conclusions was that ditchwater showed high levels of GenX and PFOA. This might be a potential concern for livestock drinking from that ditchwater. At that moment no firm conclusions were drawn regarding the transfer of GenX and PFOA to milk and edible tissue of livestock consuming this ditchwater.
The Office for Risk Assessment and Research (BURO), has requested the Front Office Food and Product Safety to address three questions related to the transfer of GenX and PFOA in ditchwater to lactating cows and sheep. A fourth question related to the transfer of GenX and PFOA in silage to lactating cows was added at a later stage to this request.

Questions
BuRO has asked the following questions with respect to the transfer of GenX and PFOA in ditchwater and silage to lactating cows and/or sheep.
1. Model the transfer of GenX and PFOA from ditchwater to edible products from lactating cows and sheep (milk and meat);
2. Estimate the intake of GenX and PFOA for consumers based on the theoretical (modelled) concentrations in milk and meat of dairy cattle and lactating sheep;
3. Calculate the possible concentrations of GenX and PFOA in ditchwater when concentrations of GenX and PFOA occur at the analytical LOQ of 0.01 ng/g in cow's milk (based on reversed dosimetry modelling).
4. Estimate the transfer of GenX and PFOA from silage to milk and meat from lactating cows and sheep.

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

Conclusions

1) A transfer model for PFOS in dairy cows was adapted for the transfer of PFOA from ditchwater to cow's milk and muscle meat. The highest intake of PFOA through the consumption of contaminated ditchwater (510 µg/day) resulted in modelled concentrations in milk and meat of 0.06 ng PFOA/g and 0.28 ng PFOA/g, respectively.
In species which show extensive renal PFOA clearance such as dairy cattle (and lactating sheep) it was assumed that comparable exposure of GenX and PFOA leads to lower concentrations of GenX in tissues and milk than PFOA.
Therefore, given the fact that the exposure to GenX from ditchwater is lower (approximately a factor 5), the expected concentration in milk and meat of dairy cattle is equal to or lower than 0.01 ng GenX/g resp. 0.06 ng GenX/g.
Only one pilot study described the kinetics of PFOA in sheep (n=2) after exposure from silage and the information does not allow us to develop a transfer model for this matrix or ditchwater in sheep. Reported transfer of PFOA to the carcass was (more or less) similar for sheep and dairy cattle, whereas transfer of PFOA to milk was possibly higher in sheep. These data need experimental confirmation before the relevance of the transfer of PFOA (and GenX) to milk and meat of sheep for human exposure can be evaluated.

2) For milk and meat of dairy cattle, the human exposure to PFOA and GenX, based on the calculated transferred concentrations of PFOA and GenX in milk and meat of dairy cattle, will be negligible and therefore do not pose a health risk. For milk and meat of sheep, more data on transfer are needed before a conclusion on human health risk can be drawn.

3) Reverse dosimetry could only be performed for PFOA. A PFOA concentration in milk at the analytical LOQ level (0.01 ng/g) leads to a modelled intake of 89 µg PFOA per dairy cow per day. This intake corresponds to a calculated PFOA concentration in ditchwater of (approximately) 810-1100 ng/L.

4) Due to the fact that (excluding other sources) the intake of PFOA through silage is 22 times lower than the intake through ditchwater it is concluded that in dairy cattle the concentrations in meat and milk will be 0.01 ng PFOA/g or 0.003 ng/g. As levels of GenX in silage were below the LOQ the transfer of GenX from silage to milk and meat of dairy cattle is considered negligible.
As mentioned under 1) calculations for the transfer of GenX and PFOA from silage to milk and meat from lactating sheep cannot yet be assessed.

Introduction

As a result of long-lasting emissions from the DuPont/Chemours chemicals company in Dordrecht, the substances GenX and PFOA have been emitted into the environment via the air and surface water. Consequently, the area in the vicinity of the factory (soil, water and vegetation) became polluted. In July 2018 the National Institute for Public Health and Environment (RIVM) has informed the city council of Dordrecht in a letter on the provisional results of research carried out in soil and irrigation water (RIVM 2018a). One of the conclusions was that ditchwater showed high levels of GenX and PFOA. This might be of concern for livestock consuming that ditchwater. In this letter it was mentioned that watering livestock at the observed high levels of GenX and PFOA in ditchwater should be avoided. In September a final report and an accompanying letter were sent to the city council of Dordrecht (RIVM 2018b). In the report, amongst others, concentrations of GenX and PFOA in ditchwater at five different locations in the vicinity of the DuPont/Chemours factory were given. At that moment no conclusions were drawn regarding the transfer of GenX and PFOA to milk and meat of livestock consuming that ditchwater.

In May 2018, the Netherlands Food and Consumer Product Safety Authority (NVWA) took samples of edible products of food producing animals (dairy products and fish) and silage in the vicinity of DuPont/Chemours company in Dordrecht and Custom Powders in Helmond. At that moment, the detection and quantification limits of the analytical method to analyse these compounds were not low enough for performing a risk assessment. In other words, if all concentrations would be below these limit values, the calculated exposure using concentrations at these limit values (worst case) would exceed the health-based guidance value. In that case, a realistic conclusion about a possible health risk cannot be drawn.

In September 2018, the FO calculated how low the LOQ for analysis of GenX and PFOA in animal products should be to be able to perform a quantitative risk assessment. Therefore, GenX and PFOA concentrations for animal products including egg, meat (beef) and cow's milk at which a high consumption of each product would result in an exposure equal to 20% of the TDI of GenX or PFOA were calculated (FO, 2018). These concentrations are referred to as '20% TDI concentrations'. No such concentrations were derived for cheese and yoghurt. The 20% TDI concentrations of GenX and PFOA in milk were respectively 0.1 and 0.06 ng/g (or ng/mL). For meat they are 1.3 ng GenX/g and 0.8 ng PFOA/g. The way these concentrations were derived is described in more detail in "Risk assessment of GenX and PFOA in food; Part 1: Toxicity of GenX and PFOA and intake of contaminated food of animal origin" (FO, 2019). If all concentrations (whether measured, modelled or reasoned) of GenX and PFOA are below these 20% TDI concentrations, a health risk can be excluded. If the concentrations are higher, an exposure assessment is required to assess possible health risks.

On 10 January 2019 the concentrations of GenX and PFOA in dairy products, fish and silage were sent to the Office for Risk Assessment and Research (BuRO). BuRO has requested the FO Food and Product Safety to address the above-mentioned questions related to the transfer of GenX and PFOA in ditchwater to lactating cows and sheep and the transfer of GenX and PFOA in silage to lactating cows and sheep. In Part 1, the risk related to the consumption of contaminated milk and meat was addressed (FO, 2019).

Toxicology

PFOA

Perfluoro-octanoic acid (CAS no. 335-67-1) (pentadeca-fluoro-octanoic acid, PFOA) and its salts are used as processing aid in the production of fluoro-elastomers and fluoropolymers, with PTFE being an important fluoropolymer. In addition, PFOA-related compounds are used as surfactant (in fire-fighting foams, wetting agents and cleaners) and for the manufacture of side-chain fluorinated polymers (used as surface finishes for textiles and apparel, leather, paper and cardboard, paints, lacquers etc.). In 2016, RIVM derived a tentative TDI (t-TDI) of 12.5 ng/kg bw per day for PFOA (Janssen, 2017). This t-TDI was based on an overall no-observed adverse level (NOAEL) of 0.06 mg/kg body weight (bw) per day for liver toxicity in a semi-chronic oral study in rats (Perkins et al. (2004).
For information on the toxicity of PFOA we refer to part 1 of the risk assessment of GenX and PFOA in food (FO, 2019).

GenX

The chemicals FRD-902 and FRD-903, referred to as “GenX chemicals”, are the main substances associated with the GenX processing aid technology that enables the production of fluoropolymers. FRD-902 is the dimer ammonium salt (ammonium-2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate; CAS no. 62037-80-3) and FRD-903 is the dimer acid (2,3,3,3-tetrafluoro-2 (heptafluoropropoxy)propanoic acid; CAS no. 13252-13-6). Under environmental and physiological conditions, such as in water or in blood, FRD-902 and FRD-903 will dissociate into the ion HFPO-DA (hexafluoropropylene-oxide dimer acid ion), which is responsible for the observed toxicological effects. In this assessment, the ion HFPO-DA is referred to as GenX. In 2017, RIVM derived a tentative TDI (t-TDI) of 21 ng/kg bw per day for GenX (Janssen, 2017). This t-TDI was based on an overall no-observed adverse level (NOAEL)[2] of 0.1 mg/kg body weight (bw) per day from a chronic oral study in rats with increased albumin/globulin ratio in serum as the critical effect (Beekman et al., 2016).
For information on the toxicity of GenX we refer to part 1 of the risk assessment of GenX and PFOA in food (FO, 2019).

Rationale of transfer assessment

This assessment focuses on the transfer of PFOA and GenX from contaminated ditch water or silage to milk and meat from dairy cattle and lactating sheep. Quantifying such transfer needs experimentally observed transfer, ideally in conjunction with a computer model describing the experimental observations (the latter enabling extrapolation of the experimental settings across dosage, matrix and exposure time duration, etc.).

PFOA: Dairy cattle (modelling approach)

As shown below an experimental study on the transfer of PFOA and its structure analogue PFOS from contaminated (grass) silage and hay to milk and meat of dairy cattle (N=6) is available. Only a PFOS transfer model based on this study is available. However, the PFOA transfer data of this study enabled the scaling of the PFOS model to PFOA (this assessment, for details, see Annex 1). This scaled PFOA model was used to quantify the transfer of this compound from ditch water or silage to milk and meat of dairy cattle (not taking into account other sources of exposure).

PFOA: Lactating sheep (experimental approach)

With regard to lactating sheep (N=2), only a pilot study on the transfer of PFOS and PFOA from contaminated (corn) silage and hay to milk and meat is available (see below). The results of this study therefore do no warrant the development of a transfer model for either of these PFASs. Furthermore, in livestock allometric scaling of PFAS kinetics does

[2] The highest dose administered in an animal study at which no adverse effects are observed

not apply (compare for example dairy cattle (Kowalczyk *et al*., (2013) with pigs (Numata *et al.* 2014). Therefore, the PFOA/PFOS transfer model in dairy cattle was not allometrically scaled from this species to sheep. In this assessment the transfer of PFOA from ditch water *or* silage to milk and meat of lactating sheep was based on the available experimental data (not taking into account other sources of exposure). The available study being a pilot, however, urges to consider the assessed transfer only as indicative.

GenX (reasoning approach)
Dairy cattle and lactating sheep show comparable, extensive renal clearance of PFOA. GenX and PFOA show comparable renal clearance in rodents and monkeys (see below). Due to lack of information on the transfer of GenX to farming animals it is assumed that dairy cattle and lactating sheep also show extensive renal clearance of GenX (for reasoning, see below).

Literature search: Transfer models
Different PubMed search strings were used to obtain information on the transfer of GenX or PFOA in dairy cows, cattle, sheep or lamb. The search initially addressed GenX and PFOA. However, as information on GenX and PFOA appeared limited the search was extended to PFOS. For dairy cattle only a transfer model for PFOS was found. In the case of lactating sheep no transfer model was found for PFOS, PFOA or GenX.

Ditch water and silage sampling of PFOA and GenX
Single samples of ditchwater were taken at five different sites within a distance (radius) of four kilometres from the DuPont/Chemours factory (Van Poll, 2018). The samples were analysed in duplicate. The average concentrations of GenX and PFOA at these sites (including their distance from the factory) are given in table 1. These results are also depicted in figure 1, which is taken from the report of Van Poll (Van Poll, 2018).

Table 1. Average concentrations of GenX and PFOA in ditchwater (in ng/L) at five different locations (codes and distances of locations are given) around Dordrecht.

Location code	Sub code	Location number	Distance (km)	GenX (ng/L)	PFOA (ng/L)
G3LOC4	WA2	8	< 1	956.5	4670
G2LOC3	WA1	6	1-2	133.5	660.5
G2LOC1	WA2	4	1-2	97.5	566
G1LOC3	WA2	3	2-3	24.5	172.5
G3LOC3	WA2	10	3-4	9.7	40.5

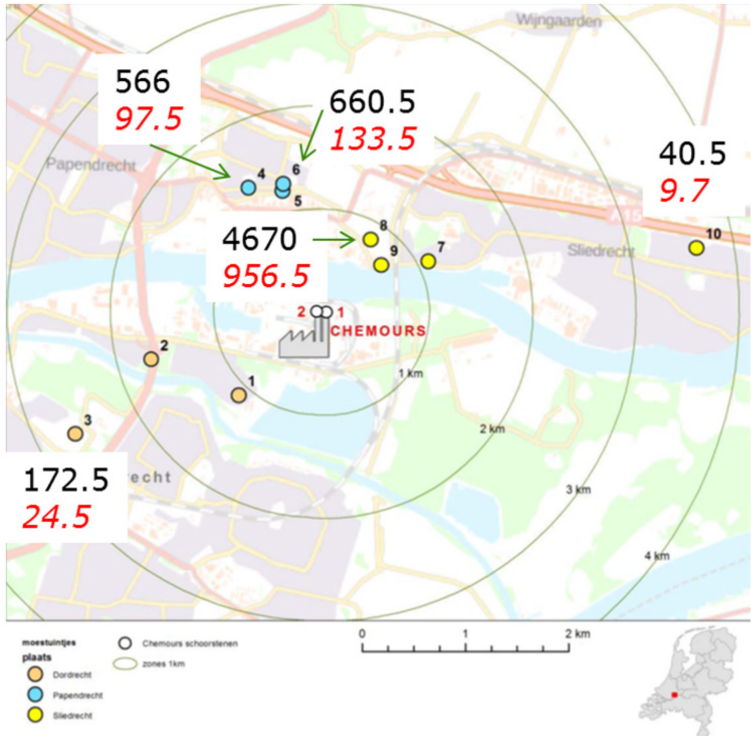

Figure 1. Average concentrations (in ng/L) of GenX (in red and italic) and PFOA (in black) in ditchwater at five different locations around Dordrecht. Ditchwater samples were taken at locations, numbered 3, 4, 6, 8 and 10.

Ten samples of silage were taken in the vicinity of Dordrecht and Helmond. No GenX could be detected in these samples (< 250 ng/kg). Only in two samples could PFOA be detected, concentrations were 540 and 600 ng/kg (measurement on basis of whole product).

Exposure scenarios

<u>Lactating cow: PFOA and GenX exposure from ditchwater or silage</u>
As shown in Table 1 the measured concentration of PFOA in ditchwater ranged from 40.5 to 4670 ng/L. Similarly, the GenX concentration ranged from 9.7 to 956.5 ng/L. Regarding the drinking water intake, lactating cows consume different volumes of drinking water per day, with (total) water intake depending on factors like ambient temperature, body weight, dry matter intake, milk production, etc. (Kume *et al.*, 2010; Meyer *et al.*, 2004; Murphy MR, 1992; National Research Council, 2001). Average drinking water consumption was used in combination with the conservative assumption that cows solely consume contaminated ditchwater. As model input for PFOA transfer model calculations, an average drinking water intake of 80 L/day for (mature) lactating cows (weighing 600 kg; milk yield: 29.5 kg day^{-1}) ranging to a maximum drinking water intake of 110 L/day (weighing 600 kg; milk yield: 35 kg day^{-1}) was used (Kume *et al.*, 2010; Meyer *et al.*, 2004; National Research Council, 2001). Then, given the maximal measured GenX and PFOA concentrations, the ditchwater intake for lactating cows is calculated at 110*956,5 ≈ 110,000 ng GenX/day respectively 110*4670 ≈ 510,000 ng PFOA/day.
PFOA, but not GenX, was detected in two samples of silage at comparable concentrations of 540 and 600 ng/kg. On average dairy cows consume 25 to 38.5 kg (grass) silage per day wet weight (Berende, 1998; Vestergren et al. 2013). As worst case (winter) scenario, the average intake of PFOA through silage for lactating cows is approximately 38.5*600 ≈ 23,000 ng PFOA/day.

Lactating cow: PFOA transfer (modelling)
Van Asselt *et al.* (2013) described a transfer model for PFOS in dairy cows. This model represents the body to be composed of blood and carcass, with PFOS being eliminated by milk clearance from the blood compartment. The model was calibrated on experimental results of a transfer experiment in which dairy cows were exposed to contaminated hay/grass silage for a 28 day period, followed by a 22 day wash-out period (Kowalczyk *et al.,* 2013; intake: 7.6 $\pm$ 3.2 µg PFOS kg bw^{-1} day^{-1}). Note that the modal calibration resulted in complete absorption of PFSA from the silage matrix. Model output consists of the PFOS concentration in the blood, carcass, milk and urine.
Next to PFOS, Kowalczyk *et al.* (2013) also provide PFOA transfer data in dairy cows. These data allowed the scaling of the PFOS model to PFOA. In concordance with the differences of PFOS and PFOA kinetics in dairy cows this scaling consisted of introducing renal clearance as the major route of excretion (while maintaining milk clearance as a minor PFOA route of excretion) in conjunction with minimizing PFOA transport from the blood to the carcass. Carcass is assumed to consist (mainly) of muscle, liver and kidney. Concentrations in muscle are expected to be half of the carcass concentrations. Furthermore, as for found for PFOS, the model applies complete absorption of PFOA from silage and, hence, ditchwater. As shown in Annex 1, Figure 4 this scaling resulted in an acceptable description of PFOA transfer data in dairy cows.

Lactating cow: PFOA reverse dosimetry (modelling back calculation from concentration in cow's milk to corresponding ditchwater concentration)
In order to calculate the possible concentrations of PFOA in ditchwater based on reversed dosimetry modelling (question 3) the above mentioned RIVM PFOA transfer model was used. The present analytical LOQ of PFOA in cow's milk (0.01 ng/g), was used as model input for the reverse dosimetry calculation. In this calculation the cow's model was used to calculate the PFOA intake through ditchwater which would lead to a milk concentration corresponding with the current analytical LOQ. Applying the above-mentioned water consumption of lactating cows, the corresponding concentration of PFOA in ditchwater can be calculated (assuming no additional exposure from other sources than ditchwater).

Lactating cow: GenX transfer (assumption)
In rodents and monkeys, GenX and PFOA preferentially partition into the blood (Butenhoff *et al.*, 2004; Gannon *et al.*, 2016). Though renal clearance is the major excretion pathway for both compounds, GenX is removed from the blood faster than PFOA (Gannon *et al.*, 2013 and references therein). From this it concluded that, due to a more efficient renal clearance of GenX, its partitioning from blood to milk and tissues is less efficient than that of PFOA. Therefore, in species which show extensive renal PFOA clearance such as dairy cattle (and lactating sheep) it was assumed that comparable exposure of GenX and PFOA leads to lower concentrations of GenX in tissues and milk than PFOA.

Lactating sheep: PFOA and GenX exposure from ditchwater and silage
Given a body weight of around 60 kg for lactating sheep (Kowalczyk *et al.*, 2012) and a daily drinking water consumption of 6 L/day, i.e. 10% of body weight, containing a (maximal) concentration of 4670 ng PFOA/L corresponds with a maximum intake of 6*4670 ≈ 28,000 ng PFOA/day, i.e. 28,000/60 ≈ 500 ng PFOA/kg bw/day (thereby excluding all other exposure sources). Similarly, taking a (maximum) concentration of 956,5 ng GenX/L corresponds to a maximum intake of 6*956,5 ≈ 5700 ng GenX/day, i.e. 5700/60 ≈ 95 ng GenX/kg bw/day.
For lactating sheep, Kemme *et al.* (2005) mention for dairy sheep a yearly meadow grass consumption of 364 kg dry matter plus 58 kg of wet weight grass silage. Assuming meadow grass to contain 40% dry matter (http://eurofins-agro.com/nl-nl/wiki/droge-stof) this yearly corresponds to 364/0,40 + 58 ≈ 970 kg wet weight grass silage, i.e. around 2.7 kg wet weight grass silage daily. Given a PFOA concentration of 600 ng PFOA/kg (see above) this results in a daily intake of 2.7 * 600 ≈ 1600 ng PFOA, i.e. 1600/60 ≈ 27 ng PFOA/kg bw/day.

Lactating sheep: PFOA transfer (experimental)
As mentioned above, no transfer model was found for PFOS, PFOA or GenX transfer in dairy cattle or lactating sheep. However, Kowalczyk *et al.* (2012) describe a pilot experiment in two sheep on the transfer of PFOA from contaminated corn silage and hay to milk (N=2) and meat (N=1). One sheep was exposed for a period of 21 day to a dose of 0.53 µg/kg bw/day, after which PFOA was measured in milk, liver, kidneys and muscle tissue. The other sheep was exposed for 21 days to 0.43 µg PFOA/kg bw/day, followed by a wash-out period of 21 days. In both sheep PFOA was mainly eliminated by renal clearance. In this sheep PFOA was completely cleared from the tissues within 10 days after the PFOA feeding period had stopped. The results of this experiment were used to estimate the transfer of PFOA and GenX from ditchwater to milk and meat, i.e. muscle tissue, and PFOA from silage.

Lactating sheep: GenX transfer (assumption)
As in dairy cattle it was assumed that comparable exposure of GenX and PFOA to lactating sheep leads to lower concentrations of GenX in tissues and milk than PFOA.

Results

Dairy cattle: PFOA

PFOA transfer from ditch water to cow's milk and meat
In the PFOA transfer model a (maximum) intake of 510 µg/day from ditchwater leads to PFOA concentrations in milk and muscle meat of 0.06 ng/g and 0.28 ng/g (carcass: 0.54 ng/g), respectively (see Annex 1). This low transfer to milk and meat is mainly due to a high renal excretion of PFOA in dairy cows. The modelled concentrations are at or lower than the 20% TDI concentration of PFOA for milk (0.06 ng/g) and meat (0.8 ng/g).

PFOA reverse dosimetry (back extrapolation from cow's milk to ditch water)
When the (analytical) LOQ of PFOA in milk (0.01 ng/g) was used in the model to back extrapolate the (theoretical) intake of dairy cows, a dose of 89 µg/day was calculated. This results in a calculated PFOA concentration in ditch water of (approximately) 1100 ng/L (for a ditch water intake of 80 L/day) and 810 ng/L (for a ditch water intake of 110 L/day). This means that whenever the concentration of PFOA in ditch water is below (approximately) 810 ng/L, it is likely that concentrations in milk will not exceed the (analytical) LOQ of PFOA in milk.

PFOA transfer from silage to cow's milk and meat
The intake of PFOA through consumption of contaminated silage of approximately 23 µg/day is much lower than the intake of PFOA through ditch water, i.e. 510 µg/day. The modelled transferred PFOA concentration to milk and meat from silage is 23/510* 0.06 ≈ 0.003 ng/g milk and 23/510*0.28 ≈ 0.01 ng/g meat.

Dairy cattle: GenX
The (maximal) exposure to GenX via ditchwater is almost five-fold lower than the (maximal) PFOA intake, i.e. 110 vs. 510 µg/day. The *assumed* GenX concentrations in milk and muscle meat then are *lower* than 0.06/5 ≈ 0.01 ng/g resp. 0.28/5 ≈ 0.06 ng/g. Note that the calculated GenX concentrations in milk and muscle meat also are lower than the GenX 20% TDI concentration of 0.1 ng/g for milk and 1.3 ng/g for meat and the the analytical LOQ of 0.1 ng/g for GenX in milk.

Sheep: PFOA and GenX
As mentioned above, one publication addressed the transfer of PFOA (and PFOS) from contaminated feed to milk and meat of two sheep (Kowalczyk, 2012).

In one sheep (sheep 2) the distribution of PFOA was experimentally determined over a 21 day exposure period at a dose of 0.53 µg/kg bw/day. In plasma PFOA increased during the first 9 days of the 21 day exposure period to a peak concentration. Milk was collected across the exposure period. After slaughter at the end of the exposure period, PFOA was measured in the liver, the kidneys and the muscle tissue, i.e. meat. As in dairy cows, the concentration of PFOA in milk was around or just above the LOD (see Table 2).

The other sheep (sheep 1) was exposed for 21 days to 0.43 µg PFOA/kg bw/day, followed by a wash-out period of 21 days. In this sheep plasma levels hardly peaked during the exposure period and were found substantially lower than in sheep 2 across the 21 day exposure period. Milk was collected across the exposure period. In this sheep PFOA was completely cleared from the plasma within 10 days after the PFOA feeding period had stopped.

Given a body weight of around 60 kg for lactating sheep (Kowalczyk *et al.*, 2012) and a daily drinking water consumption of 6 L/day, i.e. 10% of body weight, containing a (maximal) concentration of 4670 ng PFOA/L corresponds with an exposure of 6*4670/60 ≈ 500 ng PFOA/kg bw/day (thereby excluding all other exposure sources). At this exposure level the PFOA concentration in milk is expected to be approximately 0.2 - 0.7 ng/g resp. and in meat 0.2 ng/g (see Table 2).

The exposure to PFOA from silage was calculated at 27 ng PFOA/kg bw/day. The corresponding PFOA concentration range in milk is estimated to be ≈ 0.01 ng/g (27/500*0.2) – 0.04 ng/g (27/500*0.7). In meat this is approximately 0.01 ng/g (27/500*0.2).

The exposure to GenX from ditch water was calculated at 95 ng GenX/kg bw/day (approximately one fifth of the PFOA exposure). The GenX concentration in sheep milk is expected to be lower than 0.2/5 - 0.7/5 ≈ 0.04 - 0.14 ng GenX/g and in meat lower than 0.2/5 ≈ 0.04 ng/g. These concentrations are at or below the analytical LOQ of 0.1 ng/g for GenX in milk.

Table 2. Comparison of the concentration (ng/g wet weight, ± SD) of PFOA in dairy cows (N=3) exposed for 28 days to 2.0 ± 1.1 µg/kg bw/day from contaminated hay/grass silage and two lactating sheep exposed for 21 days to 0.43 µg/kg bw/day (sheep 1, S1) resp. 0.53 µg/kg bw/day (sheep 2, S2) from contaminated hay/corn silage (data taken from Kowalczyk *et al.* 2012; 2013).

Organ	Cow	Sheep
Liver	10.1 ± 1.9[3]	S2: 2.6[3]
Kidney	8.7 ± 3.9[3]	S2: 4.8[3]
Muscle	0.6 ± 0.3[3]	S2: 0.2[3]
Milk	0.14 ± 0.05[1]	S1: 0.2 ± 0.1[2] S2: 0.7 ± 0.5[2]

[1] Figure 3B, obtained from LOD: 0.1 ng/g; [2] LOD: 0.2 ng/g, average of 15 samples during the 21 day exposure period (Ranges: Sheep 1: < LOD – 0.5 ng/g; Sheep 2: < LOD – 1.3 ng/g); [3] LOD: 0.2 ng/g.

<u>Consequences for human exposure</u>
Table 3 provides an overview of the calculated PFOA transfer in dairy cattle. As shown the calculated transfer from ditch water or silage to milk remains below the 20% TDI concentration for PFOA in milk, i.e. 0.06 ng PFOA/g or meat, i.e. 0.8 ng PFOA/g.
In the case of milk in sheep the transfer calculations suggest otherwise (see above). However, in interpreting the sheep transfer calculations it should be noted that the transfer to milk was observed in only two sheep showing quite different (and partly aberrant) PFOA (and PFOS) kinetics. Secondly, taking Table 2 as a reference, the available transfer data in dairy cattle and lactating sheep indicate that PFOA transfer to organs and tissues is comparable in both species, but transfer to milk not. Regarding the latter, the limited

available data suggest a much higher transfer, i.e. up to 6 - 20 fold, of PFOA from the blood to milk in lactating sheep than in dairy cattle. For this reason it is concluded that the observed transfer of PFOA in lactating sheep to milk needs to be confirmed beyond the pilot experiment in which it was assessed in order to draw a more definitive conclusion on the relevance of such transfer for human exposure assessment. Furthermore, consumption data on sheep milk (by different population groups) in the Netherlands is not available in the Dutch Food Consumption Survey.

Table 3. Overview of calculated PFOA or GenX concentrations (ng/g) in milk and meat of dairy cattle after exposure to these chemicals via contaminated ditch water or grass silage.

Dairy cattle	PFOA		GenX	
	Milk	Meat	Milk	Meat
Ditch water	0.06[1]	0.28[1]	< 0.01[2]	< 0.06[2]
Silage	0.003[1]	0.01[1]	X[3]	X[3]

[1] Modelled; [2] Reasoned assumption, i.e. assuming less efficient transfer of GenX relative to PFOA at comparable exposure; [3] X: negligible, in other words: below LOD.

Answers
Based on the results described above the answers to the four questions asked by the Office for Risk Assessment and Research are given underneath.

Question 1
Model the transfer of GenX and PFOA from ditch water to edible products from lactating cows and sheep (milk and meat).
Answer 1
A transfer model for PFOS in dairy cows was adapted for the transfer of PFOA from ditchwater to cow's milk and muscle meat. The highest intake of PFOA through the consumption of contaminated ditchwater (510 µg/day) resulted in modelled concentrations in milk and meat of 0.06 ng PFOA/g and 0.28 ng PFOA/g, respectively.
In species which show extensive renal PFOA clearance such as dairy cattle (and lactating sheep) it was assumed that comparable exposure of GenX and PFOA leads to lower concentrations of GenX in tissues and milk than PFOA.
Therefore, given the fact that the exposure to GenX from ditchwater is lower (approximately a factor 5), the expected concentration in milk and meat of dairy cattle is equal to or lower than 0.01 ng GenX/g resp. 0.06 ng GenX/g.
Only one pilot study described the kinetics of PFOA in sheep (n=2) after exposure from silage and the information does not allow us to develop a transfer model for this matrix or ditchwater in sheep. Reported transfer of PFOA to the carcass was (more or less) similar for sheep and dairy cattle, whereas transfer of PFOA to milk was possibly higher in sheep. These data need experimental confirmation before the relevance of the transfer of PFOA (and GenX) to milk and meat of sheep for human exposure can be evaluated.

Question 2
Estimate the intake of GenX and PFOA for consumers based on the theoretical (modelled) concentrations in cow's milk and meat.
Answer 2
For milk and meat of dairy cattle, the human exposure to PFOA and GenX based on the calculated transferred concentrations of PFOA and GenX in milk and meat of dairy cattle will be negligible and therefore do not pose a health risk. For milk and meat of sheep, more data on transfer are needed before a conclusion on human health risk can be drawn.

Question 3
Calculate the possible concentrations of GenX and PFOA in ditchwater when concentrations of GenX and PFOA occur at the current limit of quantification of GenX and PFOA in cow's milk (based on reversed dosimetry modelling).
Answer 3
Reverse dosimetry could only be performed for PFOA. A PFOA concentration in milk at the analytical LOQ level (0.01 ng/g) leads to a modelled intake of 89 µg PFOA per dairy cow per day. This intake corresponds to a calculated PFOA concentration in ditchwater of (approximately) 810-1100 ng/L.

Question 4
Estimate the transfer of GenX and PFOA in silage to milk and meat from lactating cows and/or sheep.
Answer 4
Due to the fact that (excluding other sources) the intake of PFOA through silage is 22 times lower than the intake through ditchwater it is concluded that in dairy cattle the concentrations in meat and milk will be 0.01 ng PFOA/g or 0.003 ng/g. As levels of GenX in silage were below the LOQ the transfer of GenX from silage to milk and meat of dairy cattle is considered negligible.
As mentioned under 1) calculations for the transfer of GenX and PFOA from silage to milk and meat from lactating sheep cannot yet be assessed.

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Annex 1. Transfer models for contaminants in dairy cows: PFOS/PFOA

1. Introduction

In dairy cows PFOS and PFOA show quite different kinetics. For example, in the case of PFOS, milk is found the major route of excretion, with urinary excretion being negligible. Milk clearance however does not prevent PFOS accumulation in the blood and the carcass (as represented by muscle, liver and kidney), with the concentration in blood ≈ liver > kidneys >> muscle>> milk. In contrast, PFOA is only marginally detected in milk, i.e. levels just up to twofold above the LOD (0.1 µg L^{-1}). Levels in tissues were negligible when compared to an equal PFOS dosing. PFOA excretion occurs mainly via the urine with concentrations in urine >> plasma >> milk (Kowalczyk *et al.*, 2013).

The modeling of PFOS has been addressed before (Van Asselt *et al.*, 2013 and specifications herein). Here the basics of the PFOS model are summarized and its scaling to PFOA is described.

2. PFOS transfer model

The PFOS transfer model for dairy cows describes the uptake of PFOA from a feed matrix into the (free) PFOS fraction of blood serum. From circulating blood PFOS may be distributed into the cow's carcass or cleared towards bound serum PFOS, which in turn is cleared into the milk or urine (Van Asselt *et al.*, 2013). The model contains six unknown parameters, i.e. the fraction PFOS absorbed from hay/grass silage feed matrix, the free → bound clearance in the serum, the bound serum → milk clearance, the bound serum → urinary clearance, the serum flow-rate to the carcass and the serum-carcass partition coefficient. Analogous to Derks *et al.* (1993) the modeled cow's net body weight was set at 600 kg, the liver percentage of net body weight at 1.9%, the kidney fraction of net body weight at 0.3%, the blood volume fraction of net body weight at (9.3%) and the muscle fraction of net body weight at 35%. The carcass PFOS concentration was calculated as the weighted mean of the muscle, liver and kidney concentrations.

The PFOS transfer model was calibrated/verified on the basis of experimental results of Kowalczyk *et al.*(2013). In this study dairy cows (Holstein Friesian, body weight: 583 kg; N=6) were continuously exposed to hay-grass silage obtained from contaminated farmland for 28 days (upload phase, for intake data see Figure 1, N=3) or for 28 days followed by a wash-out period of 22 days (N=3). During the upload phase the overall average was 7.6 $\pm$ 3.2 µg kg bw^{-1} day^{-1}. As shown in Figure 1 for PFOS and Figure 3 for PFOA the intake during the 28 day upload phase showed a relative high intake between day 8 to 14, probably reflecting a quite high variability in the contamination level of different farmland batches. The experimental results of the exposure + wash out period for 3 cows were used to estimate unknown model parameters, whereas the results of the upload phase for the other 3 cows were used for validation purposes.

Of the six unknown parameters three, i.e. the fraction PFOS absorbed from hay/grass silage feed matrix and the milk and urinary clearances could unconditionally be identified. The remaining three parameters, i.e. the free → bound clearance in the blood, the blood flow-rate to the carcass and the blood-carcass partition coefficient appeared conditionally identifiable (for details, see Van Asselt *et al.*, 2013). As shown in Van Asselt *et al.* (2013, Figures 2 and 3, corresponding model specifications: see Table 1) the model clearly indicated PFOS to accumulate in blood serum, milk and carcass, with urinary excretion being negligible, eventually leading to a "steady state" situation (see Figure 2). Note that, as expected for bioaccumulating compounds, the time course of the accumulation does not visually reflect the time course of the daily intake.

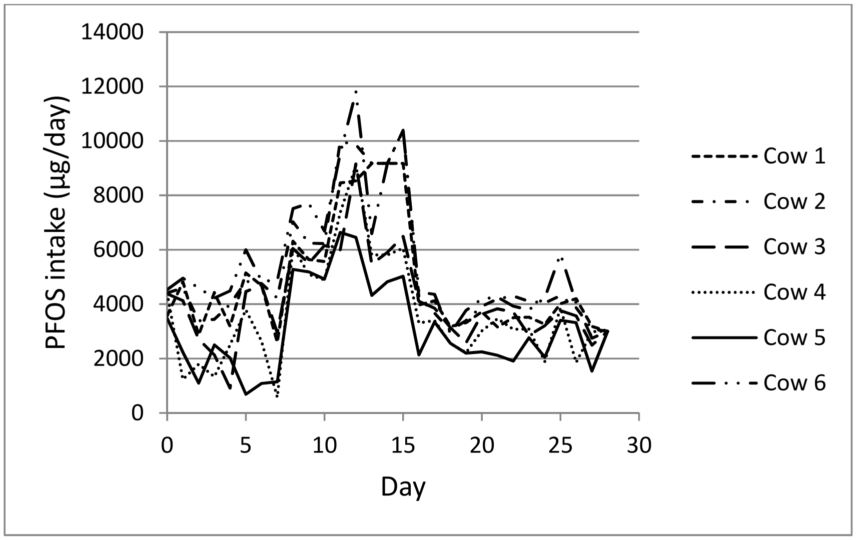


Figure 1. The daily intake (µg/day) of PFOS from contaminated hay-grass silage for a period of 28 days in dairy cows (individual data corresponding with Kowalczyk et al. (2013) supplied by WUR/RIKILT).

Table 1. PFOS model specifications (Van Asselt *et al.*, 2013).

Daily intake: Individual intake as specified in Kowalczyk *et al*. (2013)(µg day^{-1})	
Milk yield: Individual milk yield as specified in Kowalczyk *et al*. (2013)(L day^{-1})	
Fraction PFOS absorbed	1
$Serum_{free}$ → $Serum_{bound}$ clearance (CL_a)[1]	3.6 L day^{-1}
$Serum_{bound}$ → Milk clearance (CL_m)	0.017 L day^{-1}
$Serum_{bound}$ → Urine clearance (CL_u)	0 L day^{-1}
Carcass ↔ $Serum_{free}$ blood flow (Q_c)	13.4 L day^{-1}
$Serum_{free}$ -carcass partition coefficient (P_c)	28

[1] nomenclature as in Van Asselt *et al.* (2013)

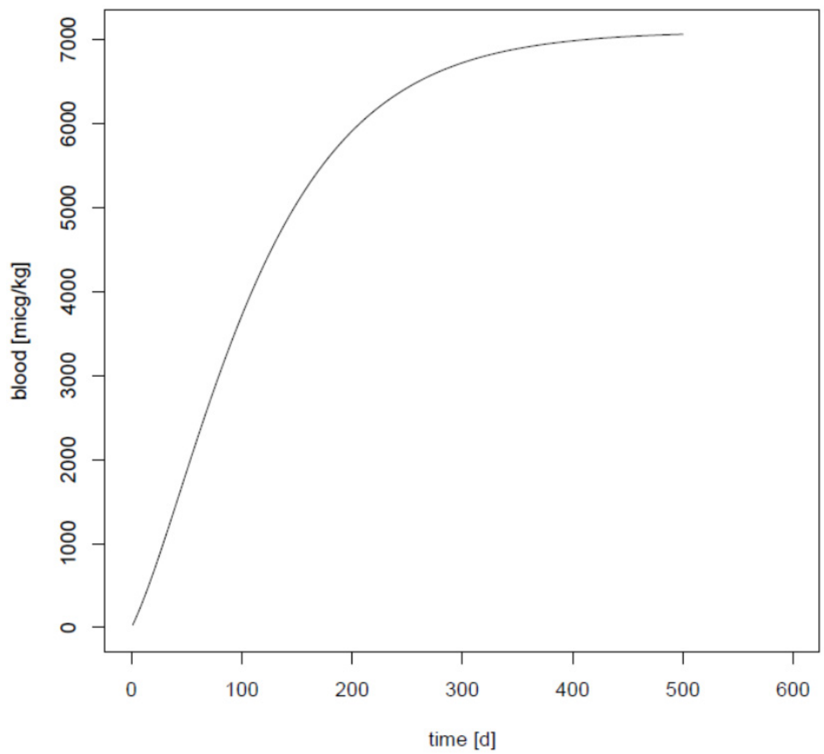


Figure 2. The time-course of the accumulation of PFOS in blood serum after continuous intake of 3000 µg day^{-1} from contaminated hay-grass silage in dairy cows. PFOS model specifications as described in Table 1.

3. PFOA transfer model

Next to PFOS the cows in Kowalczyk *et al.* (2013) were concomitantly exposed to (on average) 2.0 $\pm$ 1.2 µg PFOA kg bw^{-1} day^{-1} (see Figure 3).

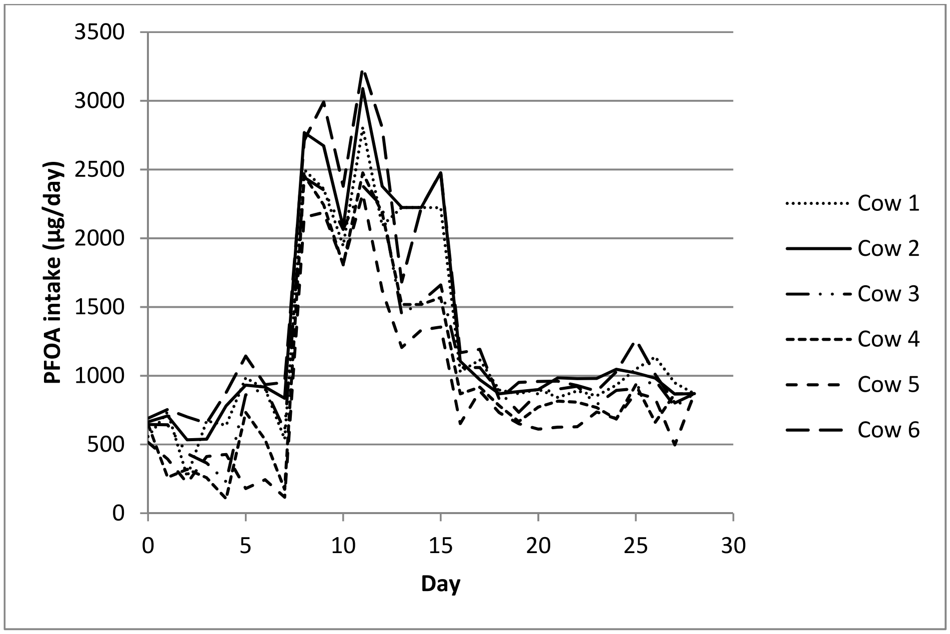


Figure 3. The daily intake (µg/day) of PFOA from contaminated hay-grass silage for a period of 28 days in dairy cows (individual data corresponding with Kowalczyk et al. (2013) supplied by WUR/RIKILT).

PFOA was excreted in the urine, with urinary concentrations ranging from 20 to 80 µg L^{-1} (to be compared with negligible PFOS urine levels). Observed levels in milk were at or just above the Limit of Detection of 0.1 µg L^{-1}. Moreover, in contrast to PFOS, the simulation characteristics closely follow PFOA intake characteristics, thereby reflecting instantaneous absorption and elimination kinetics of PFOA in dairy cows. At the end of the 28 day exposure period, levels in the liver, kidneys and muscle amounted 10.1, 8.7 and 0.6 µg kg^{-1}, corresponding with a carcass concentration around 1 µg kg^{-1} (to be compared with 295 µg PFOS kg^{-1}!). Corresponding levels in blood ranged from 9 to 16 µg L^{-1} (to be compared with around 2000 µg L^{-1} in the case of the PFOS exposure!).

Clearly, describing PFOA kinetics within the same model concept as PFOS needs re-calibration of the latter, i.e. a decrease of the transfer of PFOA from the serum to the carcass, and an increase of the mass-flow towards urinary clearance (while maintaining clearance via milk).

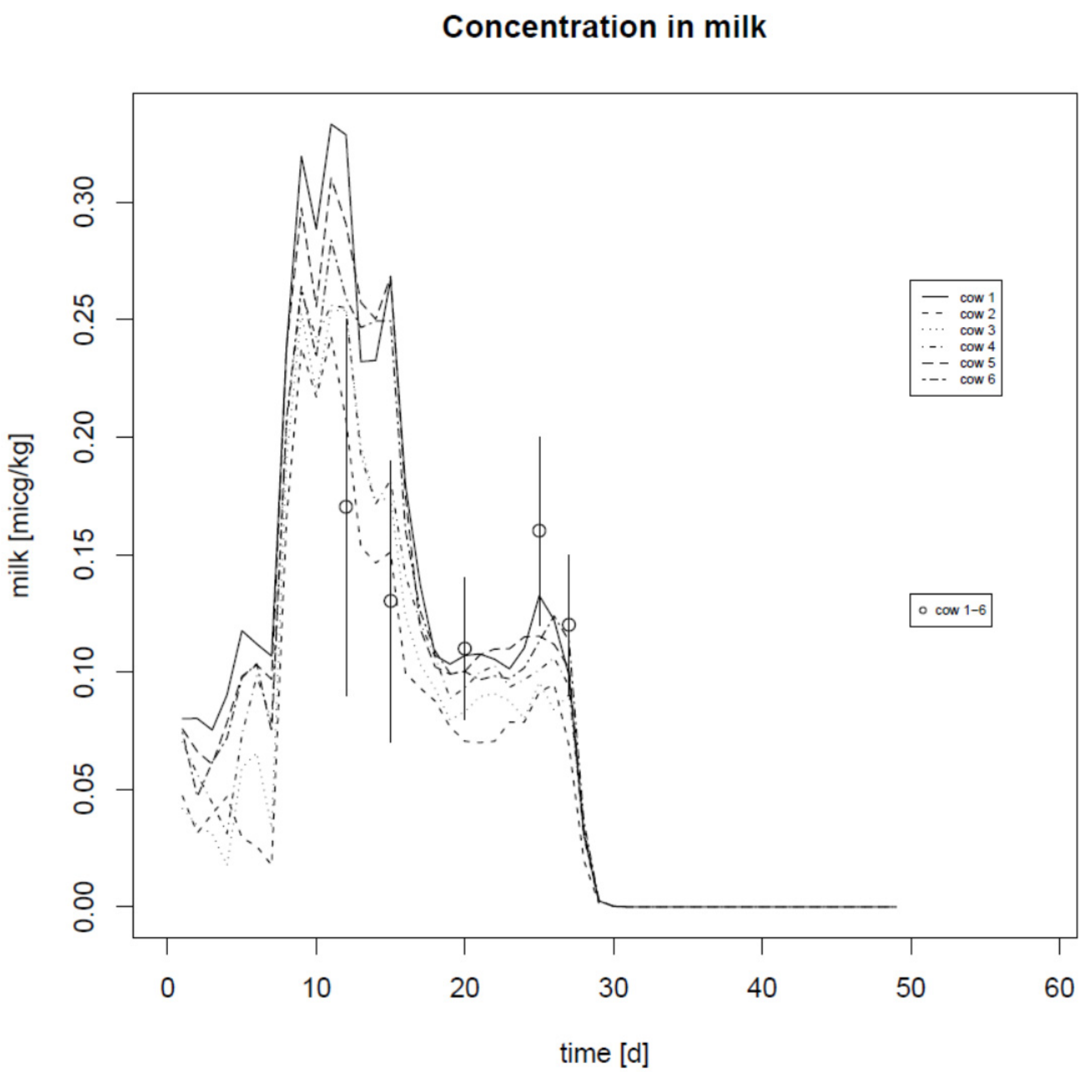
The PFOA re-calibration was performed as follows. The decrease of the transfer of PFOA to the carcass was simulated by lowering the serum carcass partition coefficient from 28 (PFOS) to 0.25 (PFOA). To increase the mass-flow towards urinary clearance the free → bound clearance in blood serum was increased from 3.6 L day^{-1} (PFOS) to 10 L day^{-1} (PFOA) and the bound blood serum → urine clearance from 0 L day^{-1} (PFOS) to 15 L day^{-1} (PFOA), thereby enabling a relative high PFOA mass flow to the urine. The corresponding bound blood serum → milk clearance was found to be 0.040 L day^{-1} (PFOA, to be compared with 0.017 L day^{-1} for PFOS)(See Table 2). As shown in Figure 4A and 4B, this re-calibration a vu led to a satisfactory description of the observed transfer of PFOA from feed to milk.

Table 2. PFOA model specifications.

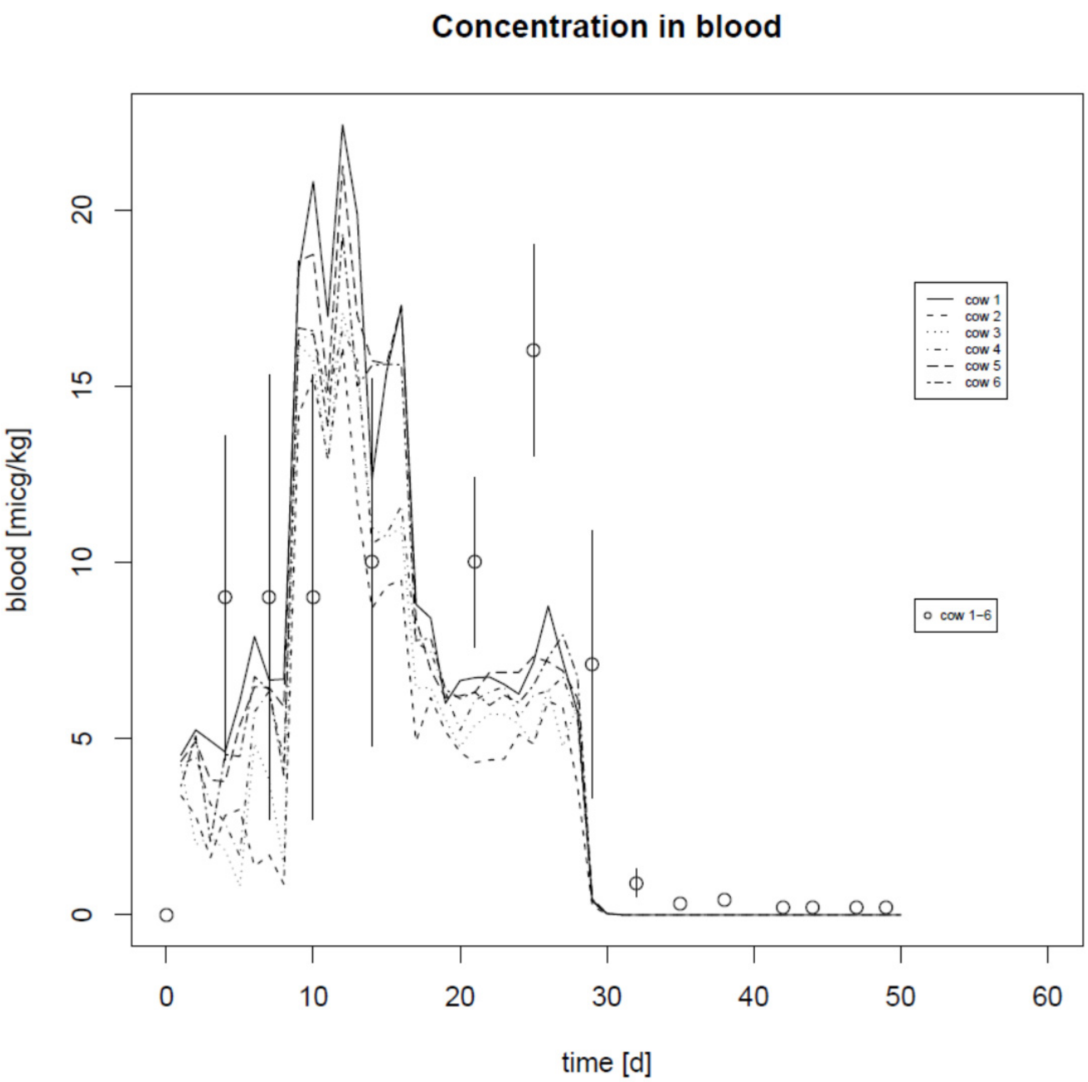
PFOA intake: Individual intake as specified in Kowalczyk *et al*. (2013)(µg day^{-1})
Milk yield: Individual milk yield as specified in Kowalczyk *et al*. (2013)(L day^{-1})

$Serum_{free}$ → $Serum_{bound}$ clearance (CL_a)	10 L day^{-1}
$Serum_{bound}$ → Milk clearance (CL_m)	0.040 L day^{-1}
$Serum_{bound}$ → Urine clearance (CL_u)	15 L day^{-1}
$Serum_{free}$ ↔ carcass partition coefficient (P_c)	0.25

A.



B.



Concentration in blood
blood [micg/kg]
time [d]
cow 1
cow 2
cow 3
cow 4
cow 5
cow 6
cow 1–6
0
5
10
15
20
0
10
20
30
40
50
60

C.

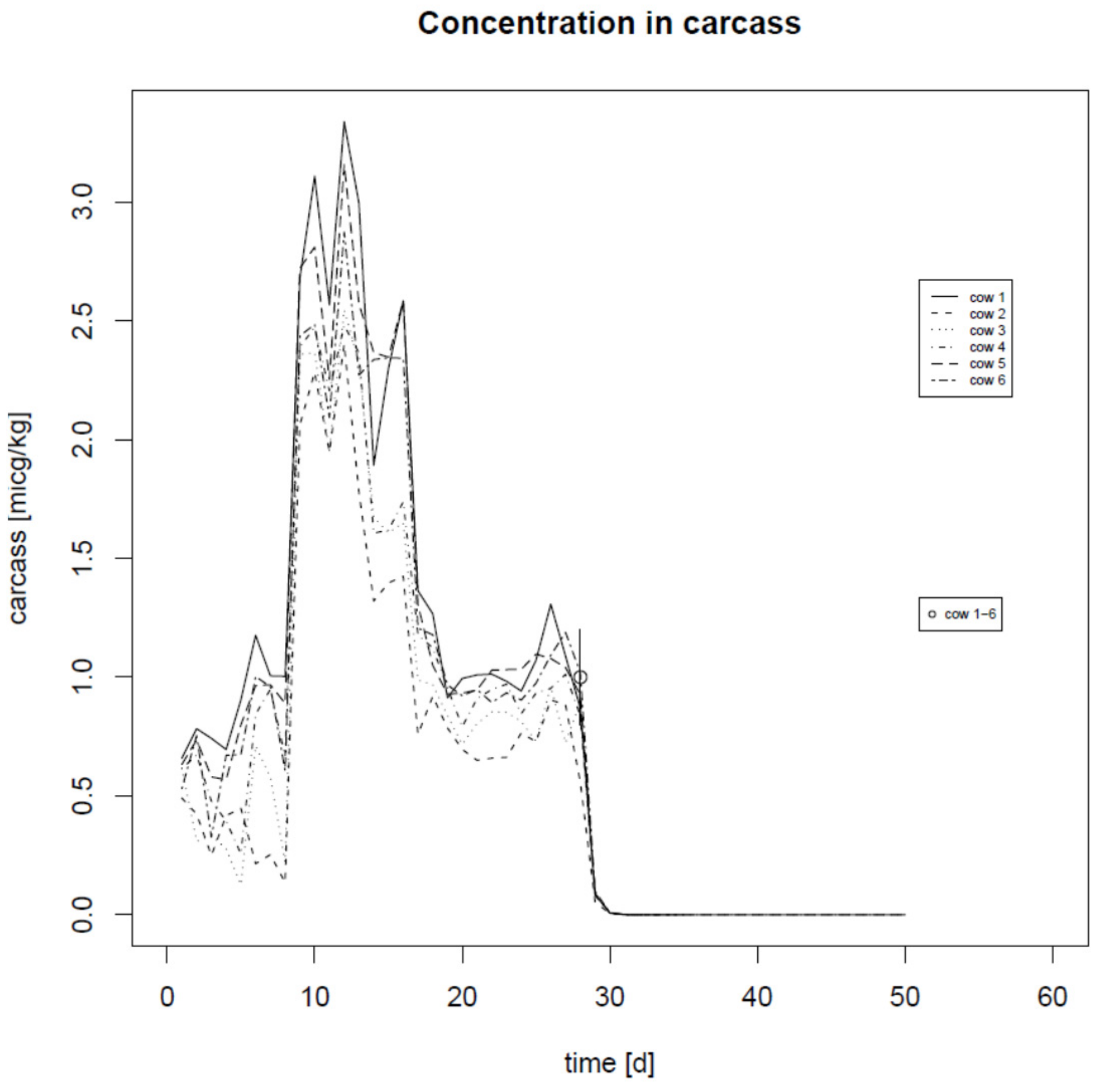


Figure 4. Model simulation of the transfer of PFOA from contaminated hay-grass silage to milk (A), blood serum (B) and carcass (C). PFOS transfer model recalibrated as specified in Table 2. Lines: cow specific individual kinetics. Symbols: experimental data (milk: upload phase, mean ± SD, to be compared with Kowalczyk et al., 2013, Figure 3 ; blood: upload + wash out phase, mean ± SD, to be compared with Kowalczyk et al., 2013, Figure 1).

4. Application of PFOA transfer model: FO question

In 2018 Dutch milk cows on average produced 28.1 kg milk day^{-1} (https://www.cooperatie-crv.nl, dd. 21-03-2019). The corresponding drinking water consumption was obtained from the study of Kume *et al.* (2010). In this study the drinking water consumption and corresponding milk yield were experimentally determined in lactating Holstein cows (body weight: 609 kg, N=16, water consumption and milk yield determined in a metabolic chamber during a 4-day time period). The average milk yield was determined at 29.5 kg day^{-1} (minimum: 21.9 kg day^{-1}; maximum: 35.3 kg day^{-1}. Similarly, the average drinking water intake was determined at 77.6 L day^{-1} (minimum: 57.0 L day $^{-1}$; maximum: 110.3 L day^{-1}).

Taking the drinking water consumption of Kume as representative for Dutch dairy cows the transfer of PFOA in drinking water in such cows to milk and meat was calculated given a daily intake of 80 liter water (corresponding with a daily milk yield of 29.5 kg day^{-1}) resp. 110 liter water (corresponding to a daily milk yield of 35 kg day^{-1}) containing 4.67 µg PFOA L$^{-1.}$, i.e. resulting in a total daily intake of 374 µg resp. 514 µg PFOA. Given 600 kg for a cow's net body weight this corresponds with an intake of 0.62 µg resp. 0.86 PFOA/kg bw/day. Note that such intake exceeds the PFOA intake of dairy cows under uncontaminated pasture conditions, i.e. around 0.6 µg per day (Vestergren *et al.*, 2013) and is somewhat lower than in Kowalczyk *et al.* (2013).
For the exposure of 0.62 µg PFOA/kg bw/day the PFOA transfer model calculates a concentration of 0.04 µg kg^{-1} for milk and 0.20 µg kg^{-1} for muscle, i.e. meat, after repeated exposure. For the 0.86 µg PFOA/kg bw/day exposure corresponding concentrations are 0.06 µg kg^{-1} for milk and 0.28 µg kg^{-1} for muscle.

Given a level of 0.01 µg L^{-1} in milk the model back-calculates a daily PFOA intake of 89.2 µg, corresponding with a ditchwater concentration ranging from 89200/110 ≈ 810 ng L^{-1} to 89200/80 ≈ 1100 ng L^{-1}, at a milk yield of 25 kg day^{-1}.

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