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Perfluoroalkylated substances in edible livers of farm animals, including depuration behaviour in young sheep fed with contaminated grass

Effrosyni Zafeiraki [a], Irene Vassiliadou [b], Danae Costopoulou [b], Leondios Leondiadis [b], Helmut A. Schafft [c], Ron L.A.P. Hoogenboom [a], Stefan P.J. van Leeuwen [a, *]

[a] RIKILT-Wageningen UR, Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands
[b] Mass Spectrometry and Dioxin Analysis Laboratory, NCSR 'Demokritos', Neapoleos 27, 15310 Athens, Greece
[c] Federal Institute for Risk Assessment (BfR), Berlin, Germany

HIGHLIGHTS

- PFOS contaminated grass causes elevated PFOS levels in sheep liver.
- Clearance occurred when uncontaminated grass was fed.
- In a market surveillance of liver samples, PFOS was the only detected compound.
- Livers of animals reared outdoors showed higher levels than those reared indoors.
- Consumption of commercially available liver is unlikely to lead to exceedance TDIs.

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ABSTRACT

Perfluoroalkylated substances (PFASs) present a potential health risk for consumers. In animals these compounds are known to accumulate in livers. In order to determine potential PFASs contamination in commercially available livers, samples from farmed sheep, horses, cows, pigs and chicken were collected from the Dutch market. PFOS was the only detectable PFAS and its concentration was higher in free ranging animals like cows and sheep. The detected levels of PFOS in the liver samples were very low (up to 4.5 ng g^{-1} ww).

To further study the kinetic behaviour in foraging animals, samples from a study in which sheep were fed with grass obtained from a river floodplain, were examined. PFOS was the only detectable PFAS in the contaminated grass pellets, showing a level of about 0.5 μg kg^{-1}. Young blackhead sheep were fed with either clean or contaminated grass for a period up to 112 days. A time-dependent increase in liver PFOS concentrations was observed from 2.4 to 10.9 ng g^{-1} ww after 8 and 112 days respectively. A time-dependent depuration was observed in livers of animals switched to clean grass after 56 days of exposure, from 9.2 to 4.7 ng g^{-1} ww after 64 and 112 days respectively. The percentage of PFOS ingested from the grass and retained in the liver was estimated to be 12% at day 56, and decreased gradually to 6% after 56 days on clean grass, showing that the decrease in levels is not only caused by an increase in liver weight.

Levels detected in commercial livers but also those in the sheep study would not lead to exceedance of the current TDI for PFOS set by EFSA. Therefore, it can be assumed that they do not present a risk for human health.

1. Introduction

Perfluoroalkylated substances (PFASs) are organic substances with chemical, thermal and biological stability, non-flammability and surface-active properties (Lau et al., 2007; Kissa, 2001). Due

* Corresponding author.
E-mail address: stefan.vanleeuwen@wur.nl (S.P.J. van Leeuwen).

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to their unique physical and chemical characteristics they have been used in many industrial and consumer applications for more than 50 years. PFASs have been detected in various matrices, including environmental (air, dust, rivers, oceans etc.) (Campo et al., 2014; Zareitalabad et al., 2013; Möller et al., 2010; Xu et al., 2013; Kubwabo et al., 2005) and biological matrices (blood, breast milk) (Kubwabo et al., 2013; Sundström et al., 2011), but also in food products (Domingo, 2012; Hlouskova et al., 2013; Klenow et al., 2013; Vassiliadou et al., 2015) and food packaging materials (Zafeiraki et al., 2014; Poothong et al., 2012; Surma et al., 2015). Their frequent detection, the environmental persistency and their ability to bioaccumulate have raised warning signs for human health. One of the most applied PFASs, perfluorooctane sulfonate (PFOS) has been added to the list of persistent organic pollutants (POPs) of the Stockholm Convention on Persistent Organic Pollutants in 2009 (Stockholm Convention on Persistent Organic Pollutants, 2009). According to toxicological studies, PFASs do not accumulate in adipose tissue like other POPs (Numata et al., 2014), but are mainly distributed to the serum, kidney and liver, with the latter showing the highest levels.

As dietary intake is considered to be the main route of human exposure to PFASs, many studies focused on the detection of PFAS levels in food items during the last years. According to two EFSA reports, edible offal and especially liver are among the most contaminated food products, both in terms of frequency and mean levels (EFSA, 2011a; 2012). According to these EFSA reports, PFAS levels were relatively high in edible offal (especially liver) from game animals, while the meat and offal from livestock animals was less contaminated. However, only a few studies provide data on PFAS levels in edible liver, either of wild or farm animals (Table S1). In addition, only a few animal studies have focused on the transfer of PFASs from contaminated feed in farm animals, in particular cows, sheep, broilers and pigs, and the consequences for food of animal origin, like milk and meat (Kowalczyk et al., 2013, 2012; Van Asselt et al., 2013; Yoo et al., 2009; Numata et al., 2014). According to these studies, PFASs and especially PFOS accumulate in animal tissues and products. In particular, PFOS levels are highest in liver, followed by kidneys and muscles. In most cases its concentration decreased after exposure was stopped, which might be due to both excretion and further growth of the animals. In contrast to animal tissues, PFOS levels initially increase in blood/plasma during a depuration period. PFOS is also excreted via milk, but overall the elimination rate of PFOS has been reported as slow (Kowalczyk et al., 2012; Van Asselt et al., 2013; Yoo et al., 2009).

In terms of sources, the environment may be more important than compound feed, meaning that edible products from foraging animals may contain higher levels than those from animals raised inside. This was recently shown for eggs from private owners (Zafeiraki et al., 2016). Among farm animals, sheep are the ones that spend in general most time outside, often the whole year round. However, the available information on kinetics of PFASs in sheep is very limited, as only one pilot study using three lactating sheep has been described (Kowalczyk et al., 2012).

In order to examine potential contamination of commercially available liver with PFASs, samples of different farm animal species were collected from local markets and slaughterhouses in the Netherlands. In addition samples were obtained from a study in which sheep were fed with grass harvested from a floodplain, initially aimed at obtaining more insight in the behaviour of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) (Hoogenboom et al., 2015). Since the grass was also found to contain PFOS, the study enabled an investigation on the relationship between the intake of PFOS and its accumulation in the liver. To our knowledge this is the first study analysing such a large number (n = 99) of liver samples from different farm animals, and also an extended study on PFOS kinetics in sheep.

2. Materials and methods

2.1. Sample collection

2.1.1. Liver samples from the market and slaughterhouses

In order to investigate potential PFASs contamination in commercially available liver, samples of different animal origin, including chicken, sheep, cow, pig and horse were purchased from local markets or obtained from slaughterhouses in the Netherlands in 2014 (see Table 1 for the number of samples per animal species). In order to avoid PFASs contamination of the sample, PFASs absorption and leaking of different plastic bottles were tested prior to the sampling process and in the end polypropylene bottles were chosen for the collection of all the samples. The plastic bottles were flushed three times with methanol (MeOH) and left to dry overnight before their use. After the collection, all the samples were transferred to the laboratory. Liver samples were homogenized and each sample was stored in a freezer (−20 °C) till the analysis.

2.1.2. Animal transfer study

Grass pellets, used in the sheep study, were previously shown to contain elevated levels of PCDD/Fs within the National Monitoring program on feed and feed ingredients in the Netherlands (Hoogenboom et al., 2015). The grass was harvested on a floodplain of the river IJssel, where the soil was reported as contaminated with relatively high levels of PCDD/Fs. As river IJssel is also known to be contaminated with PFASs, in the current study these grass samples were analysed for PFASs. Part of the contaminated grass pellets, as well as clean grass pellets were purchased from the commercial grass dryer and shipped to the Federal Institute for Risk Assessment (BfR) in Berlin, Germany, for the animal transfer study. Straw used in the study was bought by BfR from a local provider.

The details on the animal transfer study can be found elsewhere (Hoogenboom et al., 2015). In short, young blackhead sheep were purchased by BfR and transferred to animal facilities. The animal experiment was authorized by the Landesamt für Gesundheit und Soziales in Berlin with approval G0030/12, complying with the German Animal Welfare Act (Tierschutzgesetz) and supervised by the BfR institutional animal welfare officer. During the first period all the sheep were fed with clean grass pellets, while later most of the sheep received the contaminated grass pellets, starting with about 0.6 kg and increasing to 1 kg per day at the end of the up to 112 days exposure period. However, most of the sheep, after 56 days of feeding with contaminated grass pellets, were switched to clean grass pellets. Apart from grass pellets, sheep also consumed part of the straw that was used as bedding in the cages. Also some control animals, receiving clean grass, were included. During the period of this study, sheep nearly doubled their body weight from about 24 kg to 40 kg, while liver weight was slightly increased. Animals from the control group were slaughtered at day 56 and 112. From the animals fed with contaminated feed, 4 animals were slaughtered after 8, 17, 29, 56 and 112 days, in order to collect their liver and other tissues. In addition, from the animals switched to clean grass at day 56, this was performed at day 8, 15, 36 and 56 thereafter. Because part of the samples were used in another study on dioxins and PCBs (Hoogenboom et al., 2015), unfortunately not all liver samples were still available for PFAS analysis.

2.2. Chemicals

In the current study 11 PFASs: perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA),