BEHAVIOUR OF DIFFERENT PFOS-ISOMERS IN PIG TISSUES DERIVED FROM A FEEDING STUDY

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Introduction

Perfluorooctanesulfonate (PFOS) shows inert and non adhering surface properties and therefore is used in a wide range of applications. But it was observed, that PFOS is persistent and bioaccumulative as well as toxic in animal studies. In the European Union (EU), the application of PFOS is prohibited by law since 2006, but exemptions are granted (Dir. 2006/122/EC). Especially in the case of PFOS, not only the linear substances but also a considerable amount of branched isomers exist because of the production process. In most cases, PFOS is produced using electrochemical fluorination[1]. In a survey[2], a concentration dependent carry-over of PFOS and other perfluoroalkylacids (PFAA) from soil to plant was demonstrated. If plants are used as feed for food producing animals, PFAA can enter the food chain. At the Federal Institute for Risk Assessment (BfR) in Berlin feeding experiments with fattening pigs were performed to investigate the carry-over from naturally contaminated feed (grown on highly contaminated fields) into food producing animals[3]. Plasma and urine samples were taken during the PFAA feeding period. After slaughter, samples from meat, liver and kidney were taken. Feed samples were also analyzed. At the Chemical and Veterinary Analytical Institute Muensterland-Emscher-Lippe (CVUA-MEL), a separation method for the branched isomers of PFOS was developed[4]. In this paper, the behaviour of branched PFOS-isomers in comparison to linear PFOS in pig matrices derived from the feeding study is presented. The structures and the used abbreviations of the branched isomers of interest are shown in Table 1.

Table 1: Branched PFOS isomers of interest and used abbreviations

Substance	Abbreviation
Perfluorooctanesulfonate	n-PFOS
Perfluoro-1-methylheptane sulfonate	1m-PFHpS
Perfluoro-2-methylheptane sulfonate	2m-PFHpS
Perfluoro-3-methylheptane sulfonate	3m-PFHpS
Perfluoro-4-methylheptane sulfonate	4m-PFHpS
Perfluoro-5-methylheptane sulfonate	5m-PFHpS
Perfluoro-6-methylheptane sulfonate	6m-PFHpS
Perfluoro-5.5-dimethylhexane sulfonate	5,5dm-PFHxS
Perfluoro-4.4-dimethylhexane sulfonate	4,4dm-PFHxS

Materials and methods

Animal studies

Eight sows, eight barrows and eight boars were fed with PFAA-contaminated feed for 21 days. All pigs were slaughtered after these 21 days.

Sample analysis

Reagents: Native and ^{13}C-labeled PFOS were purchased from Wellington Laboratories, USA. Methanol absolute, acetonitrile, formic acid (99 %) and ammonium acetate (all ULC/MS) were purchased from Biosolve, The Netherlands. Sodium acetate anhydrous p.a. was obtained from Merck, Germany. Pepsine (from porcine

gastric mucosa) was purchased from Sigma-Aldrich, Germany. Water was double distilled by using the distillation unit 2001/2 from GFL.
Apparatus: HPLC-MS/MS: Agilent 1290 SL LC/ Agilent 6460 Triple Quadrupole LC/MS.
Sample preparation: PFOS was determined in plasma based on a method published by Kärrman et al. (2005)[5]. Internal standard solution (^{13}C-PFOS) and 4 ml of a mixture of formic acid 99 % / water (1:1) were added to 1 ml sample. The solution was sonicated for 15 minutes. After sonication, 3 ml water was added and after centrifugation the samples were purified and concentrated using solid phase extraction (SPE) on an OasisWAX (60 mg/3 ml) column. The conditioning-, washing- and eluting steps were described previously[6]. The SPE-columns were each preconditioned with 2 ml 0.1 % NH_4OH in methanol, methanol and water. After the transfer of the samples onto the weak anion exchanger, the SPE-columns were each washed with 2 ml 0.025 mol/l sodium acetate (pH 4) and methanol and subsequently put under vacuum suction until dryness. Elution was performed with 2 ml 0.1 % NH_4OH in methanol.
Muscle tissue and organs were hydrolysed using pepsine. Internal standard solution (^{13}C-PFOS) and 20 ml of a pepsine solution (1 g/100 ml; pH 2 – 2.5) were added to 1 - 3 g of the grinded samples. The samples were incubated at 37 °C for 12 hours. After incubation, the samples were heated up to > 90 °C for 10 minutes. After addition of 20 ml methanol, the samples were sonicated for 15 minutes. After a centrifugation step, the samples were purified and concentrated using SPE on an OasisWAX (150 mg/6 ml) column. The conditioning-, washing- and eluting steps were performed in the same way as described for plasma with the exception that each step was performed with 4 ml instead of 2 ml.
Feed samples were extracted using methanol. Internal standard solution (^{13}C-PFOS) and 20 ml methanol were added to 1 - 2 g of the dried, grinded samples. After shaking at room temperature for 15 minutes and sonication for 1 h, the samples were centrifuged. 10 ml of the supernatant were diluted with 10 ml water. After adjustment of the pH-value to 5 – 5.5, the samples were purified and concentrated using SPE on an OasisWAX (150 mg/6 ml) column. The conditioning-, washing- and eluting steps were performed in the same way as described for plasma. For conditioning, washing and eluting 4 ml were used.
After the elution step and addition of 30 µl glycerol as a keeper, all sample solutions were evaporated under a gentle stream of nitrogen. After reconstitution with a mixture of methanol (40 %) and water (60 %), the samples were measured using HPLC-MS/MS. Quantification was done by using isotope dilution analysis. ^{13}C-labelled linear PFOS was used as internal standard for all isomers of interest.
HPLC-MS/MS Analysis: A small column (Eclipse Plus, C18, 3.5 µm, 4.6 x 30 mm Agilent Technologies) was placed as a pre column between purge valve and autosampler to separate background PFOS from the analytes of the samples. Also an in-line filter (replacement frits 4.6 mm, 0.2 µm Agilent Technologies) between autosampler and column was used to filter remaining particles from the samples. An injector program was used to minimize a potential cross-contamination from heavily contaminated samples as far as possible.
*HPLC-Parameters: S*eparation was performed on an Agilent 1200 SL HPLC-System. A PRONTOSIL 200-3-C30 3.0 µm column (250 x 3 mm) from Bischoff Chromatography was the appropriate column to separate the individual analytes. The column temperature was held at 50 °C. Seven mM ammonium acetate adjusted to pH 7.5 was used as solvent A and methanol was used as solvent B. The flow rate was 0.3 ml/min.; Gradient: 45 % B (10 min. hold), 45 % B to 63 % B (35 min.), 5 min equilibration. 15 µl of the extract was injected into the HPLC-MS/MS system. The total run time was 50 minutes.
*Source parameters:*MS/MS-detection was performed with an Agilent 6460 triple quadrupole mass spectrometer equipped with an electrospray interface (ESI) operating in the negative ion mode. Gas temperature: 300 °C; gas flow: 4 l/min; sheath gas temperature: 350 °C; sheath gas flow: 10 l/min; nebulizer: 43 psi; capillary 3000 V (negative); nozzle voltage: 0 V. MRM settings for PFOS-isomers are published elsewhere[4,7].

Results and discussion
The individual PFOS-isomers were analyzed in the feed that was used in the feeding experiment with the pigs. In plasma-, urine-, meat- and organ samples derived from pigs of the feeding study, the individual PFOS-isomers were also analysed. The contribution of the individual PFOS-isomers in relation to total PFOS in feed and animal tissues was compared to each other in order to learn more on their behaviour. Because of the fact, that only semi-quantitative standard substances (concentration ± 20 %) were available for the branched isomers, absolute concentrations of the isomers are not shown. The fractions of all PFOS-Isomers in relation to total PFOS in feed used in the feeding study is shown in Table 2.

Table 2: Fractions of all PFOS-isomers in relation to total PFOS in feed [%]

n-PFOS	1m-PFHpS	2m-PFHpS	3m-PFHpS	4m-PFHpS	5m-PFHpS	6m-PFHpS	4,4dm-PFHxS	5,5dm-PFHxS
72.9 ± 0.6	1.4 ± 0.4	0.8 ± 0.1	3.9 ± 0.8	4.0 ± 0.3	5.1 ± 0.6	11.4 ± 0.5	0.1 ± 0.01	0.4 ± 0.04

In the analyzed feed, the amount of the branched PFOS-isomers in relation to total PFOS is about 30 %. If PFOS is produced using electrochemical fluorination, it is described that about 30 % of the produced PFOS is branched[1]. Therefore, it can be stated, that the results are conclusive.
In Figure 1, the fractions of the different PFOS-isomers in relation to total PFOS and the corresponding standard deviations are shown in feed, meat, liver and kidney for comparison. The numbers of analyzed samples are listed in Table 3. Because of the fact that no differences between sows, barrows and boars could be observed, the mean of all pigs were taken.

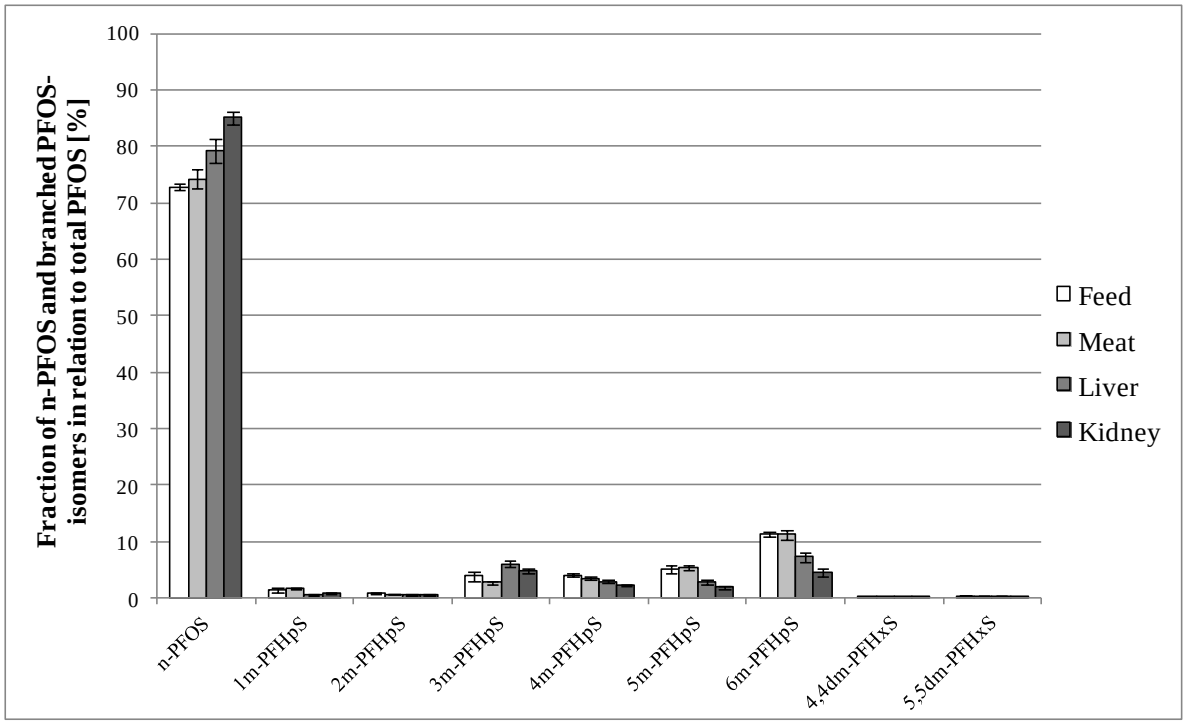

Figure 1: Fractions of the different PFOS-isomers in relation to total PFOS and corresponding standard deviations in feed, meat, liver and kidney. The numbers of the analyzed samples are listed in Table 3.

The fraction of n-PFOS in relation to total PFOS is the highest in kidney (85 ± 1 %) followed by liver (79 ± 2 %). The fractions of n-PFOS in relation to total PFOS is similar in meat and in feed (72 - 76 %). The contributions of 4m-PFHpS, 5m-PFHpS and 6m-PFHpS to total PFOS are higher in feed in comparison with liver and kidney. These results indicate that n-PFOS accumulates preferentially in liver and kidney as opposed to branched PFOS-Isomers, like 4m-PFHpS, 5m-PFHpS and 6m-PFHpS.
The results in blood and urine were not as clear as in organs, probably caused by the very low PFOS-concentrations in urine. The fraction of n-PFOS in relation to total PFOS in urine (50 ± 7 %) is the lowest compared to the fractions of n-PFOS in relation to total PFOS in feed (73 ± 1 %) and plasma (61 ± 3 %) as well as in meat and organs (see above). Because of the high standard deviations in the case of the branched PFOS-isomers, no clear conclusion could be drawn. A very large fraction of 4,4dm-PFHxS in comparison to other matrices in relation to total PFOS was determined in urine (3.5 %) for example with a standard deviation of 3.3 %.

For a better overview, the fractions of n-PFOS and the sum of branched PFOS-isomers in relation to total PFOS in the different matrices are shown in Table 3.

Table 3: Fractions of n-PFOS and the sum of branched PFOS-isomers in relation to total PFOS in the different matrices

	Fractions of n-PFOS in relation to total PFOS [%]	Fractions of the sum of branched PFOS-isomers in relation to total PFOS [%]	n
Feed	**72.9**	**27.1**	**17**
Standard deviation	0.6	0.6	
Plasma	**61.1**	**38.9**	**36**
Standard deviation	2.6	2.6	
Urine	**49.6**	**50.4**	**26**
Standard deviation	6.5	6.5	
Meat	**74.3**	**25.7**	**6**
Standard deviation	1.7	1.7	
Liver	**79.4**	**20.6**	**6**
Standard deviation	2.1	2.1	
Kidney	**85.1**	**14.9**	**6**
Standard deviation	1.2	1.2	

The data shown in Table 3 provide an indication that branched PFOS-isomers are eliminated faster via urine than n-PFOS. The fraction of n-PFOS in relation to total PFOS is lower in urine than in feed even if considering the standard deviations. This assumption is supported by the results in organs. Here, the fractions of n-PFOS in relation to total PFOS are higher than in feed even taking into account the standard deviations. Although these results are only indications it seems that the branched PFOS-isomers behave in another way than the linear PFOS in the animal body.

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