Biochemical Effect Evaluation of Perfluorooctane Sulfonic Acid–Contaminated Wood Mice (Apodemus sylvaticus)

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Wood mice (Apodemus sylvaticus) were captured at Blokkersdijk, a nature reserve in the immediate vicinity of a fluorochemical plant in Antwerp, Belgium, and at Galgenweel, 3 kilometers farther away. The liver perfluorooctane sulfonic acid (PFOS) concentrations in the Blokkersdijk mice were extremely high (0.47–178.55 µg/g wet weight). Perfluororonanoic, perfluorodecanoic, perfluoroundecanoic, and perfluorodecanoic acids were found sporadically in the liver tissue of the Blokkersdijk mice. The liver PFOS concentrations at Galgenweel were significantly lower than those at Blokkersdijk (0.14–1.11 µg/g wet weight). Further results suggest sex independence of the liver PFOS levels, increased levels of PFOS bioaccumulation in older mice, and maternal PFOS transfer to the young. Several liver end points were significantly elevated in the Blokkersdijk mice: liver weight, relative liver weight, peroxisomal β-oxidation activity, microsomal lipid peroxidation level, and mitochondrial fraction protein content. For the mitochondrial fraction catalase activity, no significant difference between locations was found. The liver weight, relative liver weight, and liver microsomal lipid peroxidation level increased significantly with the liver PFOS concentration. No indications for PFOS-mediated effects on the serum triglyceride, cholesterol, or potassium levels were obtained. The liver PFOS concentration was negatively related to the serum alanine aminotransferase activity. Key words: alanine aminotransferase, bioaccumulation, microsomal lipid peroxidation, PFOS, relative liver weight, wood mouse. Environ Health Perspect 112:681–686 (2004). doi:10.1289/ehp.6479 available via http://dx.doi.org/[Online 20 January 2004]

Anthropogenic perfluorinated acids and related perfluorinated compounds were only recently shown to be present in a great diversity of aquatic wildlife species and fish-eating mammals and birds. In these animals, perfluorooctane sulfonic acid (PFOS) was demonstrated to be the predominant perfluorinated pollutant for which concentrations up to 3.68 µg/g liver tissue in top predators have been reported. Even at remote locations, perfluorochemicals are present in animal tissues, but the levels are usually higher in more populated and industrialized regions (Giesy and Kannan 2001; Kannan et al. 2001a). Although the available literature provides only little information on perfluorochemical distribution in terrestrial mammalian species, available data show that these chemicals might be widespread in the terrestrial mammalian fauna. PFOS has been detected in livers of polar bears (0.18–0.68 µg/g wet weight), minks (0.97–3.68 µg/g wet weight), and river otters (0.034–0.99 µg/g wet weight) (Giesy and Kannan 2001; Kannan et al. 2002c).

A discrepancy exists between the scarcity of information on the presence and distribution of perfluorochemicals in terrestrial mammalian wildlife species and the relatively larger number of reports on in vivo toxicologic effects of perfluorochemicals assessed under laboratory conditions in mammalian species. Known in vivo effects of perfluorochemical exposure are an increase of the relative liver weight in the rat, mouse, and cyномolgous monkey and the induction of peroxisomal fatty acid β-oxidation and effects on several biochemical end points related to oxidative stress in the rat and mouse (Ikeda et al. 1985, 1987; Permadi et al. 1992, 1993; Sohlenius et al. 1993). Other documented effects are the induction of hypolipemia in the rat, mouse, and cyномolgous monkey (Haughom and Spydevold 1992; Lau et al. 2001; Seacat et al. 2002) and the inhibition of gap junction intercellular communication (Hu et al. 2002) and effects on carboxyl-esterase expression in the rat (Derbel et al. 1996). Developmental and maternal effects in the rabbit, rat, and mouse (Lau et al. 2001; York et al. 2000) and promotion of carcinogenesis in the rat (Abdelatif et al. 1990) have also been reported.

The aim of the present study was to evaluate the effects of PFOS exposure in wood mice (Apodemus sylvaticus) under field conditions. For the latter purpose, animals were trapped at a nature reserve next to a fluorochemical production plant and a location away from this potential pollution source. The liver concentrations of PFOS and some perfluorinated fatty acids were measured to establish possible differences in exposure between both locations. Afterward, biologic and biochemical effect end points were studied. Therefore, the liver weight, relative liver weight, liver peroxisomal fatty acid β-oxidation activity, degree of microsomal lipid peroxidation, and total protein content and catalase activity in the liver mitochondrial fraction were assessed. To study the hypolipemic effect, the serum triglyceride and cholesterol levels were measured. The serum alanine aminotransferase (ALT) activity and the serum potassium level were assessed as general markers for monitoring possible hepatic damage (Morgan et al. 2002) and renal failure (Vricella et al. 1992), respectively. The relationship between the individual PFOS levels and the latter end points was assessed. Potential age and sex effects on the PFOS bioaccumulation and the different end points were taken into account.

Materials and Methods

Sampling. In September 2002 wood mice (Apodemus sylvaticus) were captured at Blokkersdijk (n = 21) and Galgenweel (n = 21), located in the city of Antwerp, Belgium (Figure 1). Both areas are artificial sand dune habitats with willow (Salix spp.) groves. Sherman live traps were set up at dusk. The trapped animals were brought to the laboratory the next morning. Upon arrival, the animals were anesthetized with ether, and blood was taken using the retroorbital puncture method. Serum was prepared by centrifugation at room temperature (4,000 rpm, 5 min) and frozen in liquid nitrogen. After sacrificing the animals, sex, body, and liver weight were determined. The eye lenses were collected and fixed in 10% formaldehyde. The liver was dissected, weighed, and stored in liquid nitrogen for further analysis.

Age determination. The eye lenses were dried at 80°C for 24 hr and immediately weighed with an accuracy of 0.1 mg. The age of the animals (expressed in days) was calculated using the equation exp{(weight of both lenses in milligrams + 15.213)/6.568} (Vandorpe and Verhagen 1979).
Liver biochemical assays. Liver samples were homogenized on ice in 0.25 M sucrose with an MSE 150 W ultrasonic disintegrator (MSE Scientific Instruments, Sussex, UK). Mitochondrial and microsomal fractions were prepared from liver homogemate in 0.25 M sucrose according to Meijer et al. (1987). In the final step of the microsomal fraction preparation, the sucore was washed away with 0.15 M KCl. The catalase activity in the mitochondrial fraction was measured fluorometrically by a coupled reaction measuring resorufin formation (λex 540 nm, λem 590 nm) with the Amplex Red Catalase Kit (Molecular Probes, Leiden, The Netherlands). The peroxisomal β-oxidase activity was also measured on the mitochondrial fraction using fluorometric measurement of the β-oxidation rate based on the peroxisome-linked oxidation of hydroxysterol (λex 318 nm, λem 405 nm) according to Vannes and Flatmark (1991) with minor changes. The lipid peroxidation level of the microsomal fraction was determined by assessing malondialdehyde–thiobarbituric acid complex formation fluorometrically (λex 515 nm, λem 555 nm) according to Yagi (1976) with slight modifications. The protein content of the mitochondrial and microsomal fractions was determined with the Bio-Rad Protein Assay (Bio-Rad, Munich, Germany).

Serum biochemical assays. The serum ALT activity was determined by the spectrophotometric method described by Bergmeyer et al. (1986). Cholesterol concentrations were measured spectrophotometrically at 500 nm according to Allain et al. (1974), and the triglyceride concentration was assessed spectrophotometrically at 640 nm as described by Spayd et al. (1978). The serum potassium levels were measured with an ion-selective electrode on a 9180 Electrolyte Analyzer (AVL Scientific Corporation, Roswell, GA, USA). The protein content of the serum was determined with the Bio-Rad Protein Assay.

Determination of perfluorochemical concentrations. The liver concentrations of perfluorochemicals (PFOS, PFNA, PFDA, PFHxS, PFHpA, and PFDA) were measured using a combined high-performance liquid chromatography–mass spectrometry (HPLC-MSMS) according to Giesy and Kannan (2001). HPLC was done on a CapLC system (Waters, Milford, MA, USA) connected to a Quattro II triple quadrupole mass spectrometer (Micromass, Manchester, UK). Aliquots of 5 µL were loaded on an OptiGuard C18 precolumn (10 mm × 1 mm inner diameter; Alltech, Deerfield, IL, USA). The analysis was performed on a Betasil C18 column (50 mm × 1 mm inner diameter; Keystone Scientific, San Jose, CA, USA) cross-flow rate of 40 µL/min. The mobile phase was 2 mM NH₄OAc (A) / CH₃OH (B). A gradient elution was used starting at 10% B and going to 90% B in 8 min. At 10 min the initial conditions were resumed. PFOS, PFNA, PFDA, PFHpA, and PFDA were measured under (-)electrospray ionization using the respective transitions 413→569, 463→419, 499→519, 513→469, 563→519, and 513→569. The internal standard (1H,1H,2H,2H-perfluorooctane sulfonic acid) was measured under the same conditions (427→81). The dwell time was 0.1 sec. The electrospray-capillary voltage was set at −3.5 kV, and the cone voltage was 24 V. The source temperature was 80°C. The pressure in the collision cell was 3.3 × 10⁻⁵ mm Hg (Ar). The PFOS concentrations were calculated using an unextracted calibration curve. The standard deviations of replicate analyses were maximally 17%. The detection limits are shown in Table 1.

Statistical analysis. An analysis of variance (ANOVA) was used in order to test for differences in liver PFOS concentrations, age structure, and weight among the study populations. In the second instance, we carried out a general linear model analysis (GLM) (SAS 1999) to determine whether sex and age influenced the PFOS concentrations. Sex, location, and age and the interaction between latter variables were used as independent variables and the log-transformed liver PFOS concentrations as the dependent variable. We also tested whether there was a sex, location, or age effect for the different biologic and biochemical end points using GLM. The logarithmic transformed values of the biochemical end points were considered dependent variables, whereas sex, location, and age and all interactions were considered independent variables. The relationship between the different biologic and biochemical end points and the PFOS concentrations was investigated with linear mixed model analysis (LMM) (SAS 1999). The logarithmic transformed values of the biochemical end points were considered dependent variables, whereas PFOS was used as an independent variable. Location was treated as random effect to account for potential differences among sites. For the analysis of the relationship between PFOS and liver weight, we also considered body weight as covariate in the analysis. Because graphical investigation suggested that the relationship between the hepatic PFOS concentration and the relative liver weight or the liver microsomal lipid peroxidation level was not linear, we fitted nonlinear regression equations through these data using DataFit software (Oakdale Engineering, Oakdale, PA, USA).

All statistical analyses except for the nonlinear regression analyses were performed with the PROC MIXED module (SAS 1999). We used a stepwise backward selection procedure to remove all insignificant interactions from the regression model, starting with the least significant terms. The need of the random terms was assessed with the Akaike Information Criterion (Akaike 1974). The degrees of freedom of the fixed-effects F-test were adjusted for statistical dependence using Satterthwaite formulas (Satterthwaite 1941). Variance components were estimated by restricted maximum likelihood.

Results
The PFOS liver concentrations at Blokkersdijk ranged from 0.47 to 178.55 µg/g wet weight, whereas those at Galgenweel ranged from 0.14 to 1.11 µg/g wet weight (Table 1). Mean PFOS liver concentrations at Blokkersdijk differed significantly from those at Galgenweel. The median and liver PFOS concentrations

Figure 1. Study area and sampling sites.
were also higher at Blokkersdijk. Table 1 also shows that PFNA, PFDA, PFUA, and PFDOA were sporadically detected in, respectively, 5, 10, 29, and 38% of the Blokkersdijk mice but not in the Galgenweel mice. The latter perfluorocarboxylates were generally present at detectable concentrations in those mice with the highest PFOS concentrations. PFOA was not observed above the detection limit (0.11 µg/g) in any of the mice.

Mean mouse age did not differ significantly \([F_{1,34} = 0.36, p = 0.59]\) between the Blokkersdijk population (mean ± SD, 70 ± 36 days) and the Galgenweel population (71 ± 22 days). It is therefore unlikely that the differences in PFOS liver concentration are caused by a different age structure of the two populations. This is confirmed by the GLM analysis, which shows that PFOS concentrations still differ between Blokkersdijk and Galgenweel when age and sex are included in the model (Table 2). This analysis also showed an age-related increase of PFOS liver concentration but no difference between sexes was observed. The interaction terms were not significant. Body weight did not differ significantly with the highest PFOS concentrations. PFOA was also higher at Blokkersdijk. Table 1 also shows that PFNA, PFDA, PFUA, and PFDOA were, respectively, 0.09, 0.05, 0.03, and 0.04 µg/g wet weight.

The relative liver weight, mitochondrial fraction protein content, peroxisomal β-oxidation activity, and microsomal lipid peroxidation level were significantly higher in mice from Blokkersdijk than in those from Galgenweel (Table 3). The liver weight was positively related to body weight and was higher at Blokkersdijk (0.77 ± 0.23 g) than at Galgenweel (0.69 ± 0.16 g; location: \(F_{1,33} = 11.17, p = 0.002\); body weight: \(F_{1,33} = 139.66, p < 0.001\)). The mitochondrial fraction catalase activity showed no significant location effect. The mitochondrial fraction protein content and the liver lipid peroxidation level were shown to be sex dependent (Table 3). Both end points were higher in females than in males, but the difference among the liver lipid peroxidation levels of both sexes was larger at Blokkersdijk than at Galgenweel, as indicated by the significant interaction between sex and location. None of the latter end points was related to age. The relative liver weight and the liver microsomal lipid peroxidation level showed a significant relationship with the liver PFOS concentrations (Figures 2 and 3, Table 3). Liver weight, with body weight as covariate, was also positively related to liver PFOS concentration (PFOS: \(F_{1,32.7} = 4.98, p = 0.033\); body weight: \(F_{1,32.5} = 127.36, p < 0.001\); log(liver weight) = 2.4287 + 0.000559 × PFOS + 0.02821 × body weight).

The values of the different serum end points did not differ between locations or sexes. The serum triglyceride concentration was positively related to the age (Table 4). For the serum end points, a significant positive relation with the liver PFOS concentration could be shown for the triglyceride concentration, and a significant negative relation for the ALT activity (Table 4).

### Table 1. Perfluorochemical concentrations in wood mouse liver (µg/g wet weight).

| No. | PFOS         | PFNA  | PFDA | PFUA | PFDOA |
|-----|--------------|-------|------|------|-------|
| Blokkersdijk | 1 15.34 0.27 | <LOD | <LOD | <LOD | 0.04  |
|      | 2  4.94      | <LOD | <LOD | <LOD | <LOD  |
|      | 3  2.01      | <LOD | <LOD | <LOD | <LOD  |
|      | 4  1.55      | <LOD | <LOD | <LOD | 0.04  |
|      | 5  3.71      | <LOD | <LOD | <LOD | 0.04  |
|      | 6  52.65     | <LOD | <LOD | 0.15 | 0.10  |
|      | 7  0.47      | <LOD | <LOD | <LOD | <LOD  |
|      | 8  2.35      | <LOD | <LOD | <LOD | <LOD  |
|      | 9  2.89      | <LOD | <LOD | <LOD | <LOD  |
|      | 10 96.78     | <LOD | <LOD | 0.07 | 0.21  |
|      | 11 45.66     | <LOD | <LOD | 0.13 | 0.06  |
|      | 12 98.41     | <LOD | <LOD | <LOD | 0.11  |
|      | 13 1.73      | <LOD | <LOD | <LOD | <LOD  |
|      | 14 2.10      | <LOD | <LOD | <LOD | <LOD  |
|      | 15 9.19      | <LOD | <LOD | <LOD | <LOD  |
|      | 16 25.25     | <LOD | 0.19 | 0.08 | 0.09  |
|      | 17 50.07     | <LOD | <LOD | 0.08 | 0.10  |
|      | 18 4.71      | <LOD | <LOD | <LOD | <LOD  |
|      | 19 5.06      | <LOD | <LOD | <LOD | <LOD  |
|      | 20 4.30      | <LOD | <LOD | <LOD | <LOD  |
|      | 21 178.55    | <LOD | <LOD | <LOD | <LOD  |
|      | Minimum       | 0.47 | <LOD | <LOD | <LOD  |
|      | Maximum       | 178.55| 0.27 | 0.19 | 0.15  |
|      | Mean±SD       | 26.18| 0.12 | 0.22 | 0.22  |
| Galgenweel | 22 0.37      | <LOD | <LOD | <LOD | <LOD  |
|      | 23 0.19      | <LOD | <LOD | <LOD | <LOD  |
|      | 24 0.42      | <LOD | <LOD | <LOD | <LOD  |
|      | 25 0.14      | <LOD | <LOD | <LOD | <LOD  |
|      | 26 0.15      | <LOD | <LOD | <LOD | <LOD  |
|      | 27 1.11      | <LOD | <LOD | <LOD | <LOD  |
|      | 28 0.28      | <LOD | <LOD | <LOD | <LOD  |
|      | 29 0.36      | <LOD | <LOD | <LOD | <LOD  |
|      | 30 0.87      | <LOD | <LOD | <LOD | <LOD  |
|      | 31 0.24      | <LOD | <LOD | <LOD | <LOD  |
|      | 32 0.23      | <LOD | <LOD | <LOD | <LOD  |
|      | 33 0.47      | <LOD | <LOD | <LOD | <LOD  |
|      | 34 0.23      | <LOD | <LOD | <LOD | <LOD  |
|      | 35 0.19      | <LOD | <LOD | <LOD | <LOD  |
|      | 36 0.22      | <LOD | <LOD | <LOD | <LOD  |
|      | 37 0.24      | <LOD | <LOD | <LOD | <LOD  |
|      | 38 0.36      | <LOD | <LOD | <LOD | <LOD  |
|      | 39 0.30      | <LOD | <LOD | <LOD | <LOD  |
|      | 40 0.39      | <LOD | <LOD | <LOD | <LOD  |
|      | 41 0.23      | <LOD | <LOD | <LOD | <LOD  |
|      | 42 0.30      | <LOD | <LOD | <LOD | <LOD  |
|      | Minimum       | 0.14 | <LOD | <LOD | <LOD  |
|      | Maximum       | 1.11 | <LOD | <LOD | <LOD  |
|      | Mean±SD       | 0.28 | <LOD | <LOD | <LOD  |

Abbreviations: —, Median, mean ± SD cannot be calculated if values are < LOD; LOD, limit of detection. The numbers (no.) refer to individual mice captured at Blokkersdijk (1–22) or Galgenweel (23–42). LOD values for PFNA, PFDA, PFUA, and PFDOA were, respectively, 0.09, 0.05, 0.03, and 0.04 µg/g wet weight.

*Mean liver PFOS concentrations differ significantly between Blokkersdijk and Galgenweel (ANOVA: \(F_{1,34} = 81.19, p < 0.001\)).
than do serum PFOS concentration ranges that have been reported for fluorochemical production employees in Decatur, Alabama (USA), and Antwerp, which were 0.06–10.06 µg/mL and 0.04–6.24 µg/mL, respectively (Olsen et al. 2003). A similar liver concentration range (2.00–7.29 µg PFOS/g wet weight) has also been reported in fish after an accidental release of 22,000 L fire retardant foam into a nearby creek (Moody et al. 2002). This suggests that the Blokkersdijk mice were also most probably subjected to massive perfluorochemical exposure by the fluorochemical plant nearby. PFOS was not the only perfluorinated contaminant present in the liver tissue of the Blokkersdijk mice because PFNA, PFDA, PFUA, and PFDOA could also be detected, although only sporadically. The perfluorocarboxylates were generally present at lower concentrations than was PFOS, supporting the observation that PFOS is usually the perfluorochemical that is present in the highest concentrations in animal tissues (Kannan et al. 2001a, 2002b, 2002c; Moody et al. 2002) probably because it might be a breakdown product of several perfluoroochemicals (Canadian Environmental Protection Act 1999). The perfluorocarboxylates could have the same origin as PFOS because they tend to be present in the mice with the highest PFOS concentrations. At Galgenweel, the measured perfluorocarboxylates were present at levels below the detection limit, suggesting a lower degree of perfluorinated pollution at this location. Also, the liver PFOS concentrations in mice from Galgenweel were lower (0.14–1.11 µg/g wet weight) than in mice from Blokkersdijk (0.47–178.55 µg/g wet weight), suggesting that Blokkersdijk is a hot spot for perfluorinated pollution. At present, it is difficult to evaluate or to comment on the PFOS pollution degree at Galgenweel because of a lack of reports on background PFOS tissue concentrations in wildlife rodent species in Belgium. Interestingly, a liver tissue PFOS pollution gradient was recently found in two species of fish, bib (Trisopterus luscus) and plaice (Pleuronectes platessa), in the Western Scheldt River (Hoff et al. 2003), confirming the hypothesis that a point source such as this fluorochemical plant might be an important source of PFOS (precursor) release in the environment.

The age dependence of the liver PFOS level generally found in plasma of nestlings than in adult liver tissue (Kannan et al. 2001a). The results presented do not corroborate with earlier reports on PFOS concentrations in water bird liver (Kannan et al. 2002a), river otter liver (Kannan et al. 2002c), and blood or liver of ringed and gray seals (Kannan et al. 2001b, 2002b) because no age-specific trends or differences in PFOS tissue concentrations were found in these species. The youngest animal captured at Blokkersdijk was 20 days old and had a liver PFOS concentration of 52.65 µg/g wet weight (Table 1, no. 6). Because the lactation duration for wood mice is 18–22 days (Corbet and Harris 1991), we suggest that PFOS could be transferred from the dam to the pup during pregnancy and/or lactation.

In the present study, no significant differences in liver PFOS concentration among sexes were found what is concurrent with earlier data.

The regression equation is

\[ y = 0.04901 \times x^{0.7360}, \]

where \( y \) represents the relative liver weight (g liver/g body) and \( x \) the hepatic PFOS concentration (µg/g wet weight; \( R^2 = 0.42, p < 0.001 \)).

**Table 3.** Liver end points (mean ± SD) for the different study sites.

|                  | Relative liver weight (mg liver/g body) | Mitochondrial fraction protein content (mg protein/g liver) | Peroxisomal \( \beta \)-oxidation activity \( \left( 10^{-3} \text{ nmol H}_2\text{O}_2/\text{mg protein} \times \text{min} \) | Mitochondrial fraction catalase activity \( \left( \text{nmol H}_2\text{O}_2/\text{mg protein} \times \text{min} \) | Microsomal lipid peroxidation \( \left( \text{nmol malondialdehyde/mg protein} \right) \) |
|------------------|----------------------------------------|-----------------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| **Blokkersdijk** |                                        |                                                           |                                                 |                                                 |                                                 |
| Total            | 54 ± 7                                 | 2.27 ± 0.80                                              | 93.42 ± 35.06                                   | 10.09 ± 3.78                                    | 3.68 ± 1.15                                     |
| Males            | 53 ± 8                                 | 2.07 ± 0.64                                              | 92.69 ± 40.30                                   | 10.29 ± 4.01                                    | 3.03 ± 0.52                                     |
| Females          | 56 ± 7                                 | 2.66 ± 1.01                                              | 94.88 ± 24.55                                   | 9.63 ± 3.61                                     | 5.11 ± 0.75                                     |
| **Galgenweel**   |                                        |                                                           |                                                 |                                                 |                                                 |
| Total            | 46 ± 4                                 | 1.76 ± 0.95                                              | 58.76 ± 23.23                                   | 8.08 ± 2.57                                     | 2.91 ± 0.62                                     |
| Males            | 46 ± 3                                 | 1.45 ± 0.70                                              | 63.97 ± 21.66                                   | 3.12 ± 2.18                                     | 2.82 ± 0.72                                     |
| Females          | 48 ± 5                                 | 2.34 ± 1.14                                              | 49.21 ± 24.87                                   | 6.68 ± 2.56                                     | 3.03 ± 0.47                                     |
| **GLM**          |                                        |                                                           |                                                 |                                                 |                                                 |
| Location         | \( F_{1,32} = 16.41^{***} \)           | \( F_{1,31} = 5.77^{*} \)                                | \( F_{1,31} = 11.67^{**} \)                     | \( F_{1,26} = 2.10 \)                           | \( F_{1,28} = 15.56^{***} \)                    |
| Sex              | \( F_{1,32} = 1.13 \)                   | \( F_{1,31} = 5.14^{*} \)                                | \( F_{1,31} = 0.84 \)                           | \( F_{1,26} = 2.94 \)                           | \( F_{1,28} = 13.80^{***} \)                    |
| Age              | \( F_{1,32} = 0.00 \)                   | \( F_{1,31} = 0.12 \)                                    | \( F_{1,31} = 0.56 \)                           | \( F_{1,26} = 2.23 \)                           | \( F_{1,28} = 0.13 \)                           |
| Location × Sex   | —                                      | —                                                         | —                                                | —                                                | —                                                |
| **LMM**          |                                        |                                                           |                                                 |                                                 |                                                 |
| Statistics       | \( F_{1,33,9} = 5.46^{*} \)            | \( F_{1,31,0.14} = 0.13 \)                               | \( F_{1,23,6} = 0.36 \)                         | \( F_{1,18,3} = 8.43^{**} \)                    | Log10 Y = 0.4728 + 0.0001578 \times PFOS       |
| Model            | Log10 Y = 1.6895 + 0.000554 × PFOS     | —                                                         | —                                                | —                                                | Log10 Y = 1.6895 + 0.000554 × PFOS              |

— No models were constructed if statistics were not significant. GLM analysis of the effect of sex, location, and age on the liver end points. LMM analysis of the relationship between the liver PFOS concentration (µg/g wet weight) and the liver end points.

* \( p < 0.05 \); ** \( p < 0.01 \); *** \( p < 0.001 \).
on the liver or plasma PFOS concentrations for several other species (Kannan et al. 2001a, 2001b, 2002a, 2002c). In male gray seals, however, the liver and blood PFOS concentrations were found to be significantly higher than in females (Kannan et al. 2001b, 2002b). Under controlled laboratory conditions, sex-related differences in hepatic elimination rate in rats were reported for PFOA (Vanden Heuvel et al. 1991a) and PFDA (Vanden Heuvel et al. 1991b). Also, hepatic PFOA and PFNA concentrations are markedly higher in exposed male than in female rats (Kudo et al. 2000). The present report, however, does not suggest a sex-associated difference in liver PFOS concentrations. Therefore, it might be possible that sex-specific perfluorochemical tissue elimination rates are confined only to some perfluorochemicals and/or specific rodent species such as the rat.

The observed significant positive relation between the liver PFOS content and the relative liver weight corroborates earlier work on mice orally exposed to PFOS under laboratory conditions because this exposure increased the relative liver weight significantly (Sohlenius et al. 1993).

In the rat, microsomal nicotinamide adenine dinucleotide phosphate (NADPH)-dependent lipid peroxidation increased after PFOA exposure (Kawashima et al. 1994). This is consistent with the findings in the present study, suggesting an increased oxidative stress level after PFOS exposure of wood mice in the field. However, the lipid peroxidation level in the mitochondrial fraction of mice decreased in mice exposed to PFOA under laboratory conditions (Permadi et al. 1992), but this could be caused by differences in response between the mitochondrial and the microsomal fraction.

The lack of any significant predictability of the liver PFOS content toward the peroxisomal β-oxidation activity, mitochondrial catalase activity, and total protein content in the mitochondrial fraction stands in contrast with the results of previous laboratory studies in mice (Permadi et al. 1993; Sohlenius et al. 1993). It is possible that species sensitivity differences or differences in exposure regime between these laboratory studies could (partially) explain the discrepancies between the results obtained in the field and in the laboratory. Also, other perfluorinated pollutants might affect the investigated liver end points. The presence of PFNA, PFDA, PFUA, and PFDOA clearly shows that perfluorinated compounds other than PFOS are present in the Blokkersdijk mouse liver tissues, possibly affecting the liver biochemistry and contributing to the elevated values for the liver end points observed at Blokkersdijk. Moreover, Blokkersdijk—although a nature reserve—is situated at the border of a heavily industrialized area, so it is possible that the mice are contaminated with other (nonperfluorinated) compound contributing to the observed location dependence of the liver end points (except for the mitochondrial fraction catalase activity).

It has been shown that PFOS exposure drastically decreases the serum triglyceride level in mice exposed in the laboratory (Haughom and Spydevold 1992). The present results show a significant positive relationship between the liver PFOS concentration and the serum triglyceride concentration. However, the latter relation probably does not reflect an exposure–effect relationship but most likely results from the fact that the concentrations of both liver PFOS and serum triglycerides increase with age. This is illustrated by the positive relationship between the liver PFOS concentration and the age and the positive relationship between the serum triglyceride level and the age.

Perfluorochemical concentrations required to induce triglyceride accumulation in the liver of rats are much higher than those inducing the liver peroxisomal β-oxidation (Kudo and Kawashima 2003). Haughom and Spydevold (1992) showed that a perfluorochemical-mediated decrease in serum triglyceride concentration can be paralleled by an increase in hepatic triglyceride content in the rat. Taken together, these observations illustrate that the perfluorochemical threshold concentrations in rodents might be different for the serum-triglyceride-lowering effect than for the induction of hepatic β-oxidation. This difference in threshold could be an explanation for the significantly increased peroxisomal β-oxidation activity and the lack of a serum triglyceride decrease at Blokkersdijk compared with Galgenweel.

As suggested by the absence of a significant relation between the serum cholesterol and the liver PFOS concentration, no indications for a PFOS-mediated serum cholesterol-lowering effect were found.

Serum ALT activity was shown to have a significant negative relationship with the liver PFOS concentration in the present study. This contrasts with a rat study in which PFOS exposure resulted in increased plasma and serum transaminase levels (Goldenthal et al. 1978). The reason for this discrepancy is at present not clear, but it is possible that differences in exposure route and/or animal species account for this observation. Moreover, undefined confounding factors in the present study might influence the relation between the liver PFOS concentration and the serum ALT activity.

Because no significant relation between the potassium level and the liver PFOS level was observed, PFOS probably did not induce hyperkalemia in the mice, a phenomenon that has been reported for a moribund cynomolgus monkey chronically exposed to PFOS (Seacat et al. 2002) and that is used as a marker for renal failure (Vricella et al. 1992).

Recently, the liver PFOS critical toxicity value (based on reduction in rat dam body weight under controlled laboratory conditions) was set at 14.4 µg/g (Canadian Environmental Protection Act 1999). The environmental toxicity value for mammals derived from this rat study and taking into account laboratory- and field extrapolation and within- and between-species variability was defined to be 0.144 µg/g. In the presented study, the theoretical risk quotients for wood mice (defined as the ratio of their hepatic PFOS concentration and the environmental toxicity value) exceed the value of 1 for all wood mice analyzed, suggesting that there might be a toxicologic risk associated with PFOS exposure in these animals. The PFOS-related toxicologic effects are clearly supported by the observed PFOS-exposure–related biomarker effects found in the present study. However, at present it is not

| Table 4. Serum end points (mean ± SD) for the different study sites. |
|-----------------|-----------------|-----------------|-----------------|
|                 | Triglyceride    | Cholesterol     | Potassium       | ALT             |
|                 | (mg/dL)         | (mg/dL)         | (mmol/L)        | U/g protein     | U/dL            |
| Blokkersdijk    |                 |                 |                 |                 |
| Total           | 106 ± 76        | 132 ± 30        | 4.14 ± 0.97     | 1.93 ± 0.66     | 12.5 ± 4.5      |
| Males           | 85 ± 52         | 139 ± 42        | 4.12 ± 0.65     | 1.80 ± 0.46     | 11.6 ± 3.1      |
| Females         | 148 ± 105       | 151 ± 17        | 4.17 ± 1.42     | 2.15 ± 0.94     | 14.0 ± 6.5      |
| Galgenweel      |                 |                 |                 |                 |
| Total           | 102 ± 55        | 143 ± 35        | 4.13 ± 0.65     | 2.21 ± 0.64     | 14.2 ± 3.8      |
| Males           | 108 ± 65        | 124 ± 28        | 4.17 ± 0.69     | 2.21 ± 0.64     | 14.1 ± 4.1      |
| Females         | 92 ± 33         | 145 ± 31        | 4.07 ± 0.66     | 2.21 ± 0.68     | 14.3 ± 3.6      |
| GLM             |                 |                 |                 |                 |
| Location        | f1,28 = 0.23    | f1,31 = 1.13    | f1,29 = 0.00    | f1,30 = 2.37    | f1,30 = 2.44    |
| Sex             | f1,28 = 0.00    | f1,31 = 1.44    | f1,29 = 0.00    | f1,30 = 0.01    | f1,30 = 0.00    |
| Age             | f1,32 = 6.47*   | f1,31 = 2.50    | f1,29 = 0.84    | f1,30 = 3.00    | f1,30 = 3.83    |
| LMM             |                 |                 |                 |                 |
| Statistics      | f1,25 = 6.58*   | f1,33 = 2.69    | f1,31 = 0.26    | f1,32 = 3.65    | f1,32 = 4.71*   |
| Model           | Log(y) = 1.9102 + 0.002683 x PFOS | — | — | — |

— No models were constructed if statistics were not significant. GLM analysis of the effect of sex, location, and age on the liver end points. LMM analysis of the relationship between the liver PFOS concentration (µg/g wet weight) and the liver end points. * p < 0.05.
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