Environmental Sciences Europe

RESEARCH Open Access

Per- and polyfluoroalkyl substances (PFAS) and neurobehavioral function and cognition in adolescents (2010–2011) and elderly people (2014): results from the Flanders Environment and Health Studies (FLEHS)

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Abstract

Background: PFAS are persistent, bioaccumulative compounds repelling water, oil and stains which are widely used. There is mounting evidence linking exposure to a range of adverse health outcomes including renal, hepatic, immunotoxic, reproductive, endocrine disrupting and carcinogenic effects. PFAS possibly also induce neurobehavioral and developmental effects. Within Flanders Environment and Health Studies (FLEHS) internal exposure to PFAS and relevant health effects are assessed since 2008.

Results: Adolescents 14–15 y (2010–2011) living in an industrially contaminated area (without known PFAS contamination) and adults 50–65 y (2014) randomly sampled from the general Flemish population using a stratified clustered multi-stage design, were recruited. For the adolescents perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were measured in serum, for the adults PFOS, PFOA, perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA) and perfluorobutane sulfonate (PFBS). In adolescents the Neurobehavioral Evaluation System (NES3) computerized battery of tests developed to study the neurological effects of an exposure to environmental agents was applied. The adults did the Stroop test, the NES3 Continuous Performance Test and the NES3 Digit Span Test. In adolescents sleepiness, masculinity and femininity were assessed via the Epworth Sleepiness Scale and Personal Attributes Questionnaires, respectively. In adolescents PFOA was associated with significantly increased somnolence, and PFOS with a significant inverse association with boys' femininity and with girls' masculinity. In adolescents, PFAS were also associated with a marginal decrease in sustained attention (PFOS) and cognitive performance (PFOA) and a significant decrease in short-term memory (PFOS). However, in older adults PFOS was associated with a significant increase in the capacity to pay attention and PFHxS with a significant increase in sustained attention.

Conclusion: Our observations point to neurobehavioral and cognitive effects of PFAS. The neurobehavioral effects might in part result from the changes in sex hormone levels that have been reported to be associated with internal

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exposure to PFAS. Interestingly, whereas in relation to cognition some adverse effects were recorded for adolescents, for elderly persons our observations rather suggest possible weak positive effects with respect to cognition. Our observations might be in line with the view that PFAS have many, sometimes contrasting health effects.

Keywords: PFAS, Perfluorooctane sulfonate, Perfluorohexane sulfonate, Perfluorooctanoic acid, Perfluorononanoic acid, Cognition, Somnolence, Femininity, Masculinity

Background

PFAS were synthesized over 60 years ago as a new class of surfactants repelling water, oil and stains [1]. This allowed production of innovative consumer products such textile coatings, non-stick cookware, electronics, mist suppressants, and firefighting foams [1]. Emissions during its manufacture, usage, and disposal, resulted in the widespread abundance in the environment of this group of chemicals. From a regulatory and toxicological perspective, two substances of this chemical group have received past attention, PFOS and PFOA. Nowadays also much data are published on PFHxS and PFNA.

While being considered as persistent, PFAS are fundamentally different from traditional persistent organic pollutants such as polychlorinated biphenyls (PCBs). They have no aromatic ring in their structures, do not accumulate in fatty tissues and their solubility in aqueous solution is much greater than that of polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs) [2]. PFAS are thermally, chemically, and biologically inert in many circumstances or give rise to terminally persistent degradation products [3], therefore rendering them very useful for certain industrial purposes, but also non-biodegradable and bioaccumulative in the environment and food chains. Many PFAS that leach through the soil are highly mobile in groundwater systems, while others may evaporate and disseminate via the atmosphere [4]. PFAS have been detected in serum of populations in many countries including Belgium (Flanders) [5].

As well as in the environment, PFAS can persist for a long time in the human body, posing a likely threat to human health [6]. The average half-life in humans is estimated to be 5.4 years for PFOS, 3.8 years for PFOA and 8.5 years for PFHxS [7], 3.2 years for PFNA [8]. Lower half-life values (1.77 years for PFOA, 2.87 years for PFHxS and 2.93 years for linear PFOS) have been found in a study on Swedish airport employees [9]. Concerning exposure during the susceptible stage of early life, it is known that PFAS can transfer through the human placenta and via human milk [10].

There is mounting evidence linking exposure to a range of adverse outcomes including renal, hepatic, immunotoxic, reproductive, and endocrine disrupting effects [11–15]. PFAS have carcinogenic properties [6, 16–21] and possibly induce neurobehavioral and developmental effects [14]. There is concern that fetal and childhood periods are sensitive exposure windows for adverse health outcomes of PFAS [14, 22].

In Flanders, the northern part of Belgium, the successive Flanders Environment and Health Study (FLEHS) campaigns provide data on internal exposure to pollutants and associated early biological and health effects in participants of different age groups randomly sampled from the general population. Data on the determinants of exposure to PFAS and on the concentrations measured in umbilical cord plasma and in adult serum samples from FLESHS-2 (2007–2011) and FLEHS-3 (2012–2015) and in serum samples from adolescents living in an industrially contaminated site (2010–2011) with no specific records of PFAS production were reported by Colles et al. [5]. Here we present, for adolescents from FLESHS-2 and adults from FLEHS-3, data concerning the association of internal exposure to PFAS with cognitive and neurobehavioral parameters.

Methods

Participants

Adults from the Flemish reference study FLEHS 3

In the FLEHS-3 (2012–2015) campaign, adults were recruited in all five Flemish provinces to establish reference values for Flanders [23]. PFOS, PFOA, PFHxS, PFNA and PFBS were analyzed in 209 adults (50–65 years old) sampled between May 2014 and November 2014 (FLEHS-3 ADU). A stratified clustered multi-stage design was used to obtain a sample representative of the Flemish population taking into account population density, sex, socioeconomic status and age (in the selected age range 50–65). Sampling took place in three steps: first by study area, secondly by entities, the primary sampling units (PSUs), for access to participants, and thirdly by random selection of participants in accordance with the inclusion criteria. These PSUs consisted of general practitioner offices. Within each PSU, individuals were randomly selected. The study design aimed to obtain representative study populations in terms of social class, an equal distribution of males and females and of participants over age classes. Inclusion criteria were: (1) residing in Flanders for at least 10 years, (2) giving written informed consent, and (3) being able to complete an

extensive Dutch questionnaire. For the adults, exclusion criteria were: severe kidney disease (glomerular filtration rate < 60 mL/min) and active anti-cancer therapy (chemotherapy or radiotherapy). A map of Flanders (Fig. 1) shows in which area's adult participants resided.

Adolescents from the FLEHS-2 study

During the FLEHS-2 study PFOS and PFOA were measured in 199 adolescents aged 14–15 years, recruited in the municipalities Menen and Wevelgem in the "Grensland" industrial area (FL2-ADO). See Fig. 1 for a map. They were sampled from May 2010 to February 2011. The industrial contaminated area had no specific records of PFAS production. It was considered for assessment of internal exposure to PFAS because it was home to a large-scale recycling plant of ferrous and non-ferrous metals, timber industry, pigment industry, several incinerators and, until 2005, a large-scale incinerator for municipal waste. The study area was delineated according to the predominant wind direction. More details about study design and recruitment can be found elsewhere [23–27]. Our results presented on our web site show that the adolescents participating in the present study have a higher internal exposure to some industrial pollutants (https://www.milieu-en-gezondheid.be/sites/default/files/atoms/files/Brochure%20Menen%20steunpunt%202.pdf).

The FLEHS studies were approved by the Ethics Committee of the University of Antwerp and the Antwerp University Hospital (UZA), Belgium. The dossier numbers for the different studies were, respectively, UA A08 09 (FLEHS-2 adolescents of "Grensland" industrial area) and B300201419843 for FLEHS-3 adults.

Field work and sampling

Adult and adolescent participants were examined by trained research nurses and this included administration of the neurological tests (see further), collection of blood/urine samples, measurement of the height and the weight and filling out of a questionnaire. It took about an hour. Examination of the adolescents from the "Grensland" area in FLEHS 2 occurred from the 18th of May 2010 to the 12th of February 2011. Examination of the adults participating in FLEHS 3 occurred from the 26th of May 2014 to the 18th of November 2014. Forty mL peripheral blood was collected from each adult or adolescent participant and used for several biomarker measurements included in the respective studies. Blood samples were centrifuged and/or fractionated at the local sampling center and afterwards transported to the central laboratory where they were stored at − 80 °C in a biobank within 12 h after sampling. Plasma and serum were conserved at − 80 °C. All laboratory analyses were performed on coded samples, in laboratories that met quality-control standards.

Data derived from questionnaires

Participants completed a self-reporting questionnaire on personal and lifestyle factors, including: age, country of birth of their parents, weight, height, housing, residence history, occurrence of in-house structural modifications or painting, family composition, density of nearby traffic,

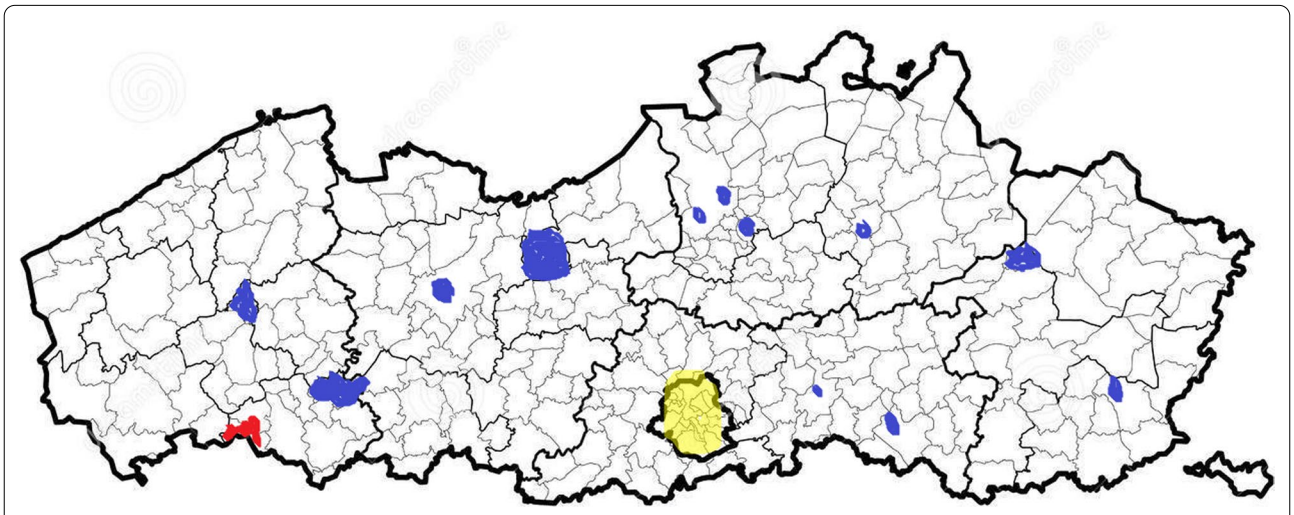

Fig. 1 Map of Flanders showing the administrative borders of municipalities. Between the most western and most eastern point: about 230 km. In red the industrial "Grensland" area (without known PFAS contamination) where the 199 adolescents participating in FLEHS 2 resided. In blue, the areas where the 209 adults participating in the FLEHS-3 campaign resided, as a random sample of the Flemish population. In yellow, the agglomeration of Brussels which is not part of Flanders and is not involved in this study

in-house use of pesticides, in-house exposures to pollutants and chemicals, sports, hobbies, contact with animals, smoking and consumption of alcohol, health status and disease experience, occurrence of asthma or eczema or allergies among relatives.

To assess socioeconomic status (SES), two different variables were evaluated separately: equivalized household income (total monthly household income standardized according to the number of household members) and highest educational attainment of the mothers in the case of neonates, of parents in the case of the adolescents and the household in the case of the adults.

Participants also completed food frequency questionnaires in order to assess the consumption of food items as described by Colles et al. [5]. The consumption of locally produced food was also recorded. Additionally, a short questionnaire on recent exposure during the past 3 days was filled out [28, 29].

Measurement of poly- and perfluoroalkyl substances (PFAS)

PFOS, PFHxS, PFOA, PFNA and PFBS were determined in peripheral serum and cord plasma using procedures as described by Kato et al. [30]. Blank bovine serum was spiked at levels of 0.4 ng/mL for PFBS, PFHxS and PFNA as well as 4.0 ng/mL for PFOA and PFOS for calculation of the recovery. As internal standards for quantification sodium perfluoro-1-hexane [18O2]sulfonate (MPFHxS) as well as sodium perfluoro-1-[1,2,3,4-13C4]-octane sulfonate (MPFOS) and perfluoro-n-[13C8]-octanoic acid (MPFOA) and perfluoro-n-[13C9]-nonanoic acid (MPFNA) were used. The analytical method consisted of an offline protein precipitation with acetonitrile, followed by separation by HPLC and MS/MS detection. Liquid chromatography was carried out on an Agilent 1100 Series HPLC apparatus. The Agilent G 1310A was used to load the processed sample (100 ul) on a restricted access material (RAM) phase, a LiChrospher RP-8ADS (25 um) 24 × 4 mm RAM from Merck (Darmstadt, Germany) using a solution of 2 mM ammonium acetate buffer pH 4 in water (solvent A) and 2 mM ammonium acetate buffer pH 4 in acetonitrile (solvent B) (80:20,*v/v*) as the mobile phase and a flow rate of 0.3 mL/min. After this clean-up and enrichment step, we transferred the analytes after 2 min to a reversed-phase HPLC column (Luna C8 (2) 150 × 4.6 mm, 3 um particle size from Phenomenex, Aschaffenburg, Germany) in back-flush mode. Tandem mass spectrometric detection was performed on a Sciex Applied Biosystems API 3000 triple quadrupole mass spectrometer (Foster City, CA, USA) in negative ionization MS/MS mode with multiple reaction monitoring. Two specific mass transitions to determine the analytes were used. Quality controls included reagent methods blanks comprising bovine serum as well as calibration standards and quality-control samples in bovine serum (ACILA AG, Weiterstadt, Germany). Reproducibility was checked by analyzing spiked bovine serum and a native human plasma sample. Recovery rates were 102% for PFOS, 99% for PFOA, 104% for PFHxS, 95% for PFNA and 91% for PFBS. Detection limits (LOD) were calculated as three times the signal/noise ratio of the analytical background noise in the temporal vicinity of the analyte signal. The limit of quantification (LOQ) was determined as twice the LOD and was 0.3 µg/L for PFOS and PFOA in FLEHS-2 (2007–2011) and in FLEHS-3 (2012–2015) LOQ was 0.2 µg/L for PFOS, PFOA, PFHxS and PFBS, and 0.1 µg/L for PFNA. In terms of PFOS and PFOA, the only PFAS for which a round robin was available at G-EQUAS (GERMAN EXTERNAL QUALITY ASSESSMENT SCHEME for Analyses in Biological Materials www.g-equas.de), participation in the German round robin G-EQUAS was successful with deviation percentages ranging from 11.7% to 15.9% for PFOS and 0.6% to 8.2% for PFOA. To assess the comparability of measurements performed during FLEHS-2 (2007–2011) with those performed during FLEHS-3 (2012–2015), PFOS and PFOA levels were re-measured in 3 samples from the biobank of FLEHS-2 together with the samples of FLEHS-3. Deviation percentages ranged from − 7.8 to − 29.4% for PFOS and + 5.0% to + 21.5% for PFOA. So, despite being measured at points in time differing by more than 3 years, the data from FLEHS 2 and FLEHS 3 are quite comparable.

Neurobehavioral tests

In adolescents of the industrial contaminated site of FLEHS-2 sleepiness, masculinity and femininity were assessed via a questionnaire as described below. The Neurobehavioral Evaluation System (NES3) computerized battery of tests developed to study the neurological effects of an exposure to environmental agents [31, 32] were performed in the adolescents of FLEHS-2 and in the FLEHS-3 adult reference population. Lack of time to perform the Stroop test in the adolescent study and other circumstances resulted in the fact that different sets of tests were applied in the adolescent and adult studies. The adolescents performed the NES3 Continuous Performance Test, NES3 Digit–Symbol Substitution Test, NES3 Digit Span Test and NES3 Finger Tapping Test. The adults in FLEHS-3 did the NES3 Continuous Performance Test and NES3 Digit Span Test, aside from the Stroop test.

In the Continuous Performance Test, a series of letters is displayed on the screen, one at a time, and each for approximately 200 ms. The task is to immediately respond to the letter S, and not to other letters, by

pressing the spacebar. A new letter appears each 1000 ms. In total, the letter S appears 60 times. The mean reaction time for responding to the target letter in ms (CPTmean) and the number of incorrect (CPTincor) reactions were used as the measure of performance. The test evaluates sustained attention. It showed a good test–retest reliability in a group of patients directed to a neuropsychological examination [32]. In the Digit–Symbol Substitution Test (DSST), used to measure general cognitive performance, a row of 9 symbols paired with 9 digits is displayed at the top of the screen. The same 9 symbols but in a different order are shown at the bottom. When a digit is displayed, the task is to indicate the symbol, which is paired with this digit, from the bottom row. A new digit appears only after the correct symbol has been indicated. In total, 27 digits are displayed. The total time needed to complete the test measured in seconds (DSSTlat) describes the performance. The number of errors (DSSTnerr) was recorded [Letz R.: NES3 user's manual. Atlanta (GA): Neurobehavoral Systems Inc, 2000]. The Digit Span Test consists of two parts. In the first part, a subject hears a sequence of digits. The task is to reproduce them. In case of a correct answer, a one digit longer sequence is presented. In case of a mistake, a sequence of the same length is presented. When two incorrect answers in a row are given, the first part of the test finishes. The second part is the same as the first one, but the sequences are reproduced in the reverse order. Digit Span Forward (DSF) is the maximum span reproduced in the first part. Digit Span Backward (DSB) is the maximum span reproduced in the reverse order. The first part of the test assesses the working memory span. Good performance in the second part requires both the ability to hold and manipulate information. For adolescents in the FLEHS-2 industrial area, in a sub-population, the Digit Span Test was administered using computers with touch screens instead of a keyboard. In order to account for a possible effect of the way the test was administered, an indicator variable was included in the regression models for this test. In the Finger Tapping Test (FTT) a subject presses the spacebar as many times as possible during a trial of 10 s. The first part of the test consists of 4 trials with the preferred hand. The second part consists of 4 trials with the non-preferred hand. The summary measures are the total number taps with the preferred hand (FTTpref) and the total number of taps with the non-preferred hand (FTTnpref). The test measures the manual motor speed.

The Stroop test assesses the person's capacity to pay attention by changing colors and the way colors are written (selective attention domain). In this test, four buttons are displayed on the screen (yellow, red, blue, and green). During the test, the name of one of these colors appears on the screen printed in a different color than the name. The task is to touch as fast as possible the button that has the same color as the name, ignoring the color of the printed name. Before the test, eight practice trials take place followed by 48 test trials. The mean reaction time is the average time that passed between the appearance of the name and touching the correct button, expressed in ms (Strooptime). The number of errors made was also recorded (Strooper).

Additionally, in the FLEHS-2 adolescents, sleepiness during the day was evaluated based on 8 questions (high to no chance of somnolence during activities such as reading, TV watching, inactivity). The scoring was done using the Epworth Sleepiness Scale, a scale between 0 and 24 [33]. The extent to which male and female identity (masculinity or femininity) were present in the test persons was evaluated using the Personal Attributes Questionnaire as proposed by Spence and Helmreich [34], with a scoring between 0 (completely agree) and 4 (not agree at all) on 24 questions on attitudes, such as aggression, independence, emotionality, passiveness, decisiveness, self-confidence.

Statistical analysis

Biomarker values were reported if they were above a quantifiable level as determined in the laboratory. Taking into account that, for the PFASs for which associations with biological or health effects could be studied, the percentage of measurements below the LOQ was low, values below the LOQ were replaced by LOQ/2. Associations between internal exposure to PFAS compounds and parameters of biological or health effects were assessed using multiple linear regression or multiple logistic regression, taking into account predetermined confounding factors. Confounders were selected a priori based on experience in previous studies and a literature search. Potential covariates were included on the basis of mechanistic considerations or because they showed, in one of the cohorts in our study, an association ($p < 0.25$) with a dependent variable of interest. Confounders and covariates were included as continuous variables whenever continuous data were available. These potential covariates are listed in the additional file 1 Tables S1, and S2. Stepwise multiple linear or logistic regression analyses were done using R version 3.3.0 and RStudio version 1.0.136, and logistf for Firth logistic regression for associations where exposure variables showed quasi separation in the normal logistic regression. In the models the PFAS concentrations were included on the original scale, without transformation. To limit the number of independent variables in the final models, confounders were removed from the model if they had a $p > 0.5$ and subsequently covariates were removed if they had a $p > 0.05$. For each association the confounding factors

and significant covariates included in the final model are mentioned in the additional file 1 Tables S1 and S2. Continuous effect markers were ln-transformed if this normalized the distribution of the regression residuals. For linear and logistic regression, estimates and odds ratios (OR) were calculated, respectively. Estimates, OR and their 95% confidence intervals were reported for interquartile (IQR) increases in exposure.

Estimates for IQR differences in exposure, were either calculated as 'ratios' or 'differences' in the levels of effect markers, respectively, in case of ln-transformed or non-transformed effect markers, used in the regression models. To put this estimate into context, this change in the effect marker was also expressed in percentage of the observed difference in effect marker between the 25th and 75th percentile within the respective study population. Associations with a $p < 0.05$ or a $p < 0.1$ are, respectively, designated as significant or marginally significant.

Regression analyses are sensitive to influential cases, whose deletion from the dataset would noticeably change the result of the calculation. Outliers are cases with y values—in this case effect marker values—deviating from the trend. Cases have high leverage if they have extreme or unusual combinations of predictor values, here exposures, confounders or covariates. Although these are not necessarily faulty data, they lie 'far' from the other cases and are too few to develop robust models. We are aware that the exclusion of influential cases, in particular cases with high exposure values, can potentially have implications in terms of environmental justice [35], but the number of subjects in our studies was too low to address this problem. In order to get a model that was correct for the general population, it was decided to test exposure–response associations without influential outliers, employing two methods for their identification. The first was to identify outliers as cases with Studentized residuals with an absolute value larger than 3, and check which of these also had high leverage by determining if their hat value was higher than twice the average hat value of all cases in the regression. The second method was to plot the Cook's distance of each case. We decided not to work with a cut-off value for the Cook's distance since the traditional cut-off values identified either no influential cases or too many (often 5% or more). Instead, cases that had a Cook's distance clearly higher than the rest of the cases and/or higher than one of the influential cases identified using the first method were also identified as influential cases [36]. Combining these metrics allows identification of influential cases, and although the exact cut-offs to be used are as usual controversial [37], we picked conservative values that seemed to produce at most a few influential cases in our models. We present results after exclusion of influential cases. For most associations there were no or only one influential case, the maximum number of influential cases was 4 (this occurred in two associations as shown in Additional file 1: Tables S3 and S4 show results in which as well data with as without influential cases are described.

Results

PFAS serum concentrations

PFAS concentrations in cord plasma or serum of the subjects participating in the FLEHS-2 and -3 studies have been published in detail by Colles et al. [5]. A summary is provided in Table 1. At least 99.5% of the PFOA or PFOS values were above LOQ in the FLEHS-2 and FLEHS-3 studies. The same was true for PFHxS and PFNA measured in adults of FLEHS-3. For PFBS measured in adults of FLEHS-3, the detection rate was below 5%, so we did not attempt to study associations between PFBS and cognitive parameters.

Results of the neurocognitive tests

The results of the neurocognitive tests are summarized in Table 2.

Associations between PFAS serum concentrations and neurobehavioral and cognitive parameters

Several neurobehavioral or cognitive parameters were assessed on adolescents in FLEHS-2 and on adults in FLEHS-3 (see methods). Associations after removal of any influential cases are shown in Figs. 2, 3. Statistical data, also comprising all cases, can be found in the

Table 1 Summary of PFAS exposure levels measured in the adolescents (FLEHS2) and adults (FLEHS 3)

Campaign, population	PFAS µg/L	LOQ µg/L	% above LOQ	period	n	Median µg/L	P_{25} µg/L	P_{75} µg/L	P_{90} µg/L
FLEHS-2, Adolescents aged 14–15	PFOS	0.3	100	2010–2011	199	5.70	4.00	7.80	10.80
FLEHS-3, Adults aged 50–65	PFOS	0.2	100	2014	205	7.58	5.22	11.15	16.30
FLEHS-3, Adults aged 50–65	PFHxS	0.2	99.5	2014	205	1.61	1.02	2.36	3.61
FLEHS-2, Adolescents aged 14–15	PFOA	0.3	100	2010–2011	199	2.60	2.13	3.00	3.62
FLEHS-3, Adults aged 50–65	PFOA	0.2	100	2014	205	2.94	2.13	3.69	4.93
FLEHS-3, Adults aged 50–65	PFNA	0.1	100	2014	205	0.87	0.60	1.18	1.64

Table 2 Scores of neurocognitive and neurobehavioral tests performed for adolescents or adults

Campaign	Population	Effect parameter	n	Arithmetic mean	Median	P_{10}	P_{25}	P_{75}	P_{90}
FLEHS-2	Adolescents 2010–2011	CPTmean (ms)	186	411	400	363	379	435	474
		CPTincor (number)	186	5.62	5	2	3	7	11
		DSSTlat (seconds)	195	98.5	94.26	80.40	86.23	104.73	120.37
		DSSTnerr (number)	194	0.959	1	0	0	1	2
		DSF (number)	194	5.54	5	4	5	6	7
		DSB (number)	194	4.48	4.5	3	4	5	6
		FTTpref (number)	194	289	287	242	263	311	340
		FTTnpref (number)	194	260	258	222	240	278	304
		Epworth Sleepiness (Scale value)	182	5.30	5	1	3	8	10
	Male adolescents 2010–2011	Masculinity score[a]	107	2.46	2.50	1.88	2.13	2.75	3.25
	Male adolescents 2010–2011	Femininity score[a]	107	2.56	2.63	2.00	2.38	2.88	3.25
	Female adolescents 2010–2011	Masculinity score[a]	80	2.12	2.13	1.50	1.75	2.50	2.75
	Female adolescents 2010–2011	Femininity score[a]	80	3.03	3.00	2.50	2.75	3.31	3.50
FLEHS-3	Adults 2014	CPTmean (ms)	192	422	410	373	388	439	475
		CPTincor (number)	183	2.02	1	0	0	3	5
		DSF (number)	187	5.18	5	4	4	6	7
		DSB (number)	186	4.24	4	3	4	5	5
		Strooptime (ms)	198	1242	1173	984	1057	1344	1591
		Strooper (number)	198	0.626	0	0	0	1	2

[a] From personal attributes questionnaire

Additional file 1 Table S3 and Table S4. Below the main findings are summarized.

NES-battery of neurological tests

For the NES-battery of neurological tests, we observed only a few significant or marginally significant associations with the PFAS levels. In adolescents (of FLEHS-2) some of these associations pointed in the direction of decreased cognitive performance. Serum PFOA concentrations were marginally significantly associated with an increase in the number of errors (DSSTnerr) in the Digit–Symbol Substitution Test ($p=0.059$) (Fig. 2), with an interquartile increase in PFOA associated with an increase of 0.182 in the number of errors, corresponding to 18.2% of the difference between the p_{75} and p_{25} values in the number of errors observed among all the adolescents tested in FLEHS-2. PFOS concentrations were significantly associated with a decrease in the maximum span reproduced in the reverse order (DSB) in the Digit Span Test ($p=0.02$), with an interquartile increase in PFOS concentration associated with a decrease of 0.139 digits in the number of digits that could be reproduced, corresponding to 13.9% of the difference between the p_{75} and p_{25} values in the number of digits observed among all the adolescents tested in FLEHS-2 (Fig. 2), suggesting a decrease in short-term memory and capacity to hold and manipulate information. Also, PFOS concentrations were marginally significantly associated with an increase in the time needed to react (CPTmean) in the Continuous Performance Test ($p=0.073$), with an interquartile increase in PFOS concentration associated with an increases of 0.7% in the mean reaction time, corresponding to 4,6% of the increase in reaction time at P_{75} compared to P_{25} among all adolescents tested in FLEHS-2 (Fig. 3), suggesting a possible decrease in the capacity to concentrate and maintain a sustained attention.

Contrasting with the findings for adolescents, for the older adults participating in FLEHS-3, 21 of the 24 associations between PFAS serum concentrations and NES tests results pointed, non-significantly or significantly, in the direction of increased cognitive performance. Favorable associations pointing in the direction of increased cognitive performance were observed for all associations studied, except for the association of PFNA with DSF and DSB and of PFOA with DSF which were in an unfavorable direction (Fig. 2). Some of the associations were significant. Indeed, a higher PFOS serum concentration showed an inverse, thus favorable, association with the Strooptime, ($p=0.049$). An interquartile increase in PFOS internal exposure was associated with a decrease of 2.4% in the Strooptime (i.e., quicker reaction time), corresponding to 11.1% of the decrease in reaction time at P_{25} compared to P_{75} among all adults tested in FLEHS-3, suggesting an increase in the capacity to concentrate (Fig. 3).

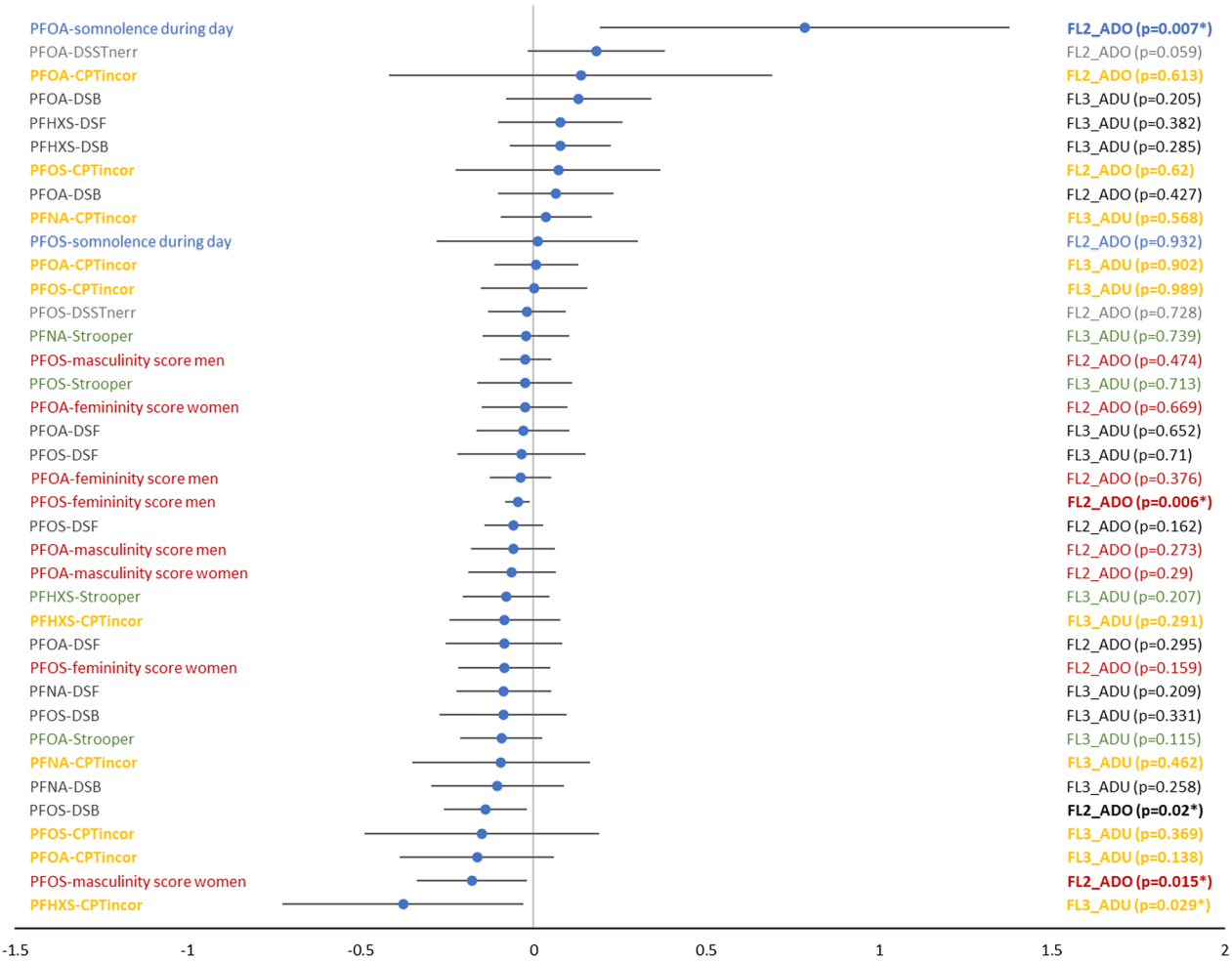

Fig. 2 Forest plot of the estimates of **neurocognitive tests** (NES3 tests, Stroop test) and **behavior** (somnolence score and femininity/masculinity score) in relation to the measured PFAS in the different FLEHS studies (with FL2 = FLEHS-2, FL3 = FLEHS-3, ADO = adolescents, ADU = adults). The estimate (95% confidence interval) is expressed as an increase or decrease in effect marker, associated with an IQR increase in exposure. Calculations based on effect marker values without logarithmic transformation. Confounders and covariates that were included at the start of the stepwise multiple regressions are mentioned in Additional file 1 Tables S1 and S2

The adults of FLEHS-3 also showed a significant inverse, favorable, association between higher PFHxS serum concentration and the CPTincor in the Continuous Performance Test ($p = 0.029$), with an interquartile increase in PFHxS concentration associated with a decrease of 0.377 errors corresponding to 12.6% of the decrease in errors between the p_{25} compared to the p_{75} values in the number of errors observed among all the adults tested in FLEHS-3, suggesting an increase in the capacity to concentrate and in sustained attention (Fig. 2).

Neurobehavioral parameters assessed for adolescents

Sleepiness during the day was assessed in adolescents of FLEHS-2. A higher PFOA serum concentration showed a significant positive association with sleepiness during the day ($p = 0.007$), with an interquartile increase in PFOA concentration associated with an increase in somnolence of 0.786 points in the Epworth Sleepiness Scale, corresponding to 15.7% of the increase between the p_{75} value compared to the p_{25} value in somnolence score observed among all the adolescents tested in FLEHS-2. For PFOS, this association was non-significant (Fig. 2). Furthermore, **masculinity and femininity** were evaluated using the Personal Attributes Questionnaire in FLEHS-2 adolescents. All 8 associations with PFOA or PFOS serum concentrations were non-significantly or significantly inverse (Fig. 2). For males the PFOS concentration showed a significant inverse association with femininity, with an interquartile increase in PFOS concentration associated with a decrease of 0.046 points in the Personal

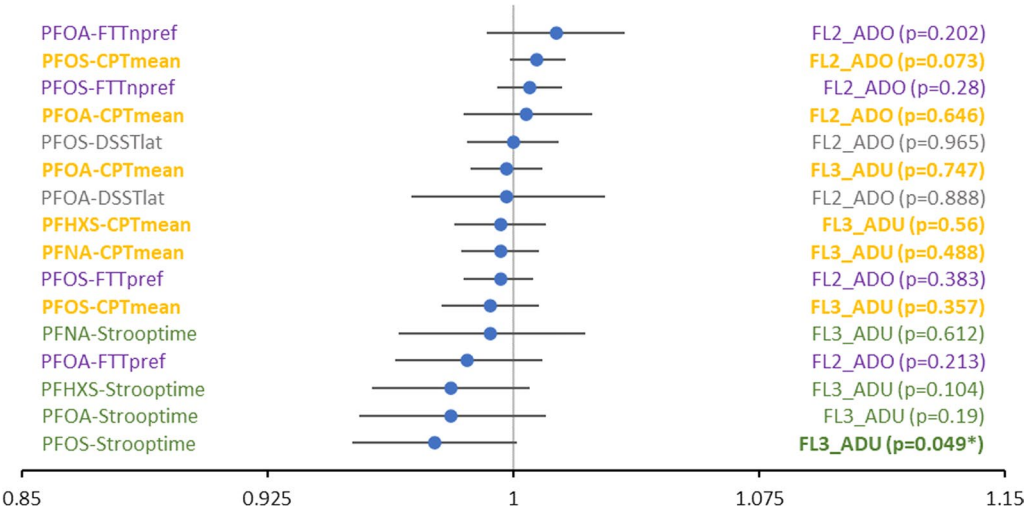

Fig. 3 Forest plot of the estimates of outcomes of the **neurocognitive tests** (NES3 tests, Stroop test) in relation to the measured PFAS in the different FLEHS studies (with FL2 = FLEHS-2, FL3 = FLEHS-3, ADO = adolescents, ADU = adults).). The estimate (95% confidence interval) is expressed for an interquartile increase in exposure (calculations on ln-transformed effect marker values). Confounders and covariates that were included at the start of the stepwise multiple regressions are mentioned in Additional file 1 Tables S1 and S2

Attributes Questionnaire score ($p = 0.006$), corresponding to 7.36% of the difference between the p_{75} value and the p_{25} value in femininity score observed among all male adolescents participating in FLEHS-2. For female adolescents the PFOS serum concentration showed a significant inverse association with masculinity, with an interquartile increase in PFOS concentration associated with a decrease in masculinity of 0.178 points in the Personal Attributes Questionnaire score ($p = 0.015$), corresponding to 23.73% of the difference between the p_{75} value and the p_{25} value in the masculinity score observed among all female adolescents participating in FLEHS-2 (Fig. 2).

Discussion

Internal exposure to PFAS in Flanders in the period of 2010–2014, covered by the studies included in this paper, were in the middle or low range compared to concentrations observed in other Western countries [5]. Differences in PFAS serum levels between our adolescent and adult cohorts were described in detail and discussed by Colles et al. [5]. As described under Methods the measurements on adolescents and adults were comparable in spite of being done at different points in time. So, in spite of the time trend towards decreasing internal exposure to PFAS in Flanders reported and discussed by Schoeters et al. [38], the serum levels measured in our adult cohort in 2014 were still higher than those measured in our adolescent cohort in 2010–2011. This is consistent with the increasing serum concentrations of PFAS with increasing age reported in several publications [39–41]. Differences between associations observed here for older adults and adolescents cannot rest on the fact that real internal exposure to PFAS of the older adults would have been lower than that of the adolescents.

Our results on the associations of PFAS levels in relation to neurobehavioral tests are in line with other contrasting and variable observations that are reported in the literature concerning the biological and health effects of PFAS.

Somnolence and gender identity

For adolescents of FLEHS-2, we observed a significant positive association between PFOA (but not PFOS) blood concentrations and somnolence during the day. In terms of gender identity as assessed through the Personal Attributes Questionnaire, a significant inverse association was seen for PFOS with femininity for boys and with masculinity for girls (Fig. 2) using the Personal Attributes Questionnaire as proposed by Spence and Helmreich [34]. We are, however, aware of the fact that this questionnaire is not able to measure the complexity of global masculinity or femininity [42] and of limitations affecting this questionnaire in terms of societal changes around gender identities [43]. We are not aware of any observations concerning PFAS and somnolence. Speculatively, it might be argued that induction of somnolence by PFAS may involve the possibility that PFAS share some physicochemical properties with fluorinated organics (such as fluorinated oxolanes and oxetanes) that were reported to produce somnolence or anesthesia [44]. To our knowledge, no associations between internal exposure to PFAS and masculinity or femininity have

been reported in the literature. However, PFAS exposure associated to sex-, age- and compound-specific changes in sex hormone concentrations has been reported by Xie et al. [45]. These changes in sex hormone concentrations might well explain the changes in gender-specific behavior we observed. In particular, a decrease in testosterone levels associated with PFOS as observed in Taiwanese girls 12–17 years of age [46], American girls 6–9 years of age [47] and American Girls aged 12–19 [45] might contribute to the quite important decrease in masculinity observed for girls. Also for boys changes in oestradiol and testosterone concentrations were reported by Nordström-Joensen et al. [48], Lopez-Espinoza et al. [47] and Xie et al. [45], which might contribute to the observed decrease in boys' femininity.

Other functions and cognition

Neonatal exposure to PFOS or PFOA caused neurobehavioral defects in adult mice [49]. Unfavorable developmental or neurobehavioral effects on humans of prenatal exposure to PFAS were reported by Donauer et al. [50] (hypotonic babies at 5 weeks of age) and Hoyer et al. [51] (hyperactivity and behavioral problems in children). However, not all studies observed adverse effects. Fei et al. [52] found, at concentrations higher than in our study, no convincing associations between developmental milestones in early childhood and levels of PFOA or PFOS measured in maternal plasma early in pregnancy. PFAS measured in cord plasma in the Netherlands, at concentrations lower than in our study, had no impact on 18-month-old children examined using the Child Behavior Checklist 1.5–5, in 'Attention Deficit Hyperactivity Disorder' scores but showed a significant inverse association with externalizing problem behavior [53]. Harris et al. [54] reported that, at concentrations higher than in our study, higher prenatal levels of some PFAS were associated with a better non-verbal IQ in children with a mean age of 7.7 years. Stein & Savitz [55] observed, in 5–18 years old children, an inverted J-shaped association between PFOA and attention deficit/hyperactivity disorder (ADHD) (small increase in prevalence for the second quartile of exposure compared with the lowest, and a decrease for the highest versus lowest quartile). Forns et al. [56] observed no association between PFOS and PFOA measured in breast milk samples one month after delivery and cognitive and psychomotor development at 6 and at 24 months and behavioral development at 12 and at 24 months. A systematic review by Roth & Wilks [57] concluded that the epidemiological evidence did not support a strong causal association between PFAS and adverse neurodevelopmental and neurobehavioral outcomes in infants and children.

Considering neurocognitive effects, rather opposite findings were seen for adolescents vs. older adults. Our results concerning unfavorable effects on cognition in adolescents—unfavorable effects on sustained attention (CPTmean) (Fig. 3), short-term memory (DSB) (Fig. 2) and cognitive performance (DSSTnerr) (Fig. 2)—are consistent with several published reports. In the Hokkaido Birth Cohort Study [58] PFOA, but not PFOS, adversely affected mental development in girls at 6 months of age. The association between maternal PFAS concentrations and early communication development in British girls at 15 and 38 months of age varied by maternal age at delivery. In daughters of younger mothers (< 25 years of age), every 1 ng/mL of PFOS was associated with a 3.82 point (95% confidence interval (CI) − 6.18, − 1.47) lower vocabulary score at 15 months and a 0.80 point (95% CI − 1.74, 0.14) lower language score at 38 months [59].

The molecular mechanisms which might contribute to neurobehavioral or cognitive effects were studied in cell lines [60] (different mechanisms for different PFAS). As reviewed by Wang et al. [61], PFAS could affect neurotransmitter concentrations, expression of neurotransmitter receptors, synaptogenesis and synaptic plasticity, induce apoptosis of neuronal cells, and alter the expression of microRNAs. As to the basic mechanisms intervening in the biological activity of PFAS, activation of PPARα, a ligand-activated transcription factor intervening in lipid homeostasis and inflammation, probably plays a role [62]. In addition, other putative mechanisms have been proposed, such as gap junctional inhibition to disrupt cell–cell communication, mitochondrial dysfunction, interference of protein binding, partitioning into lipid bilayers, oxidative stress, alterations in calcium homeostasis and related signaling pathways [63] and changes in DNA methylation [64, 65].

Our results concerning favorable effects on cognition in older individuals of 50–65 y—favorable effects on the capacity to concentrate (Strooptime) (Fig. 3) and to sustain attention (CPTincor) (Fig. 2)—were consistent with several publications. A cross-sectional study using data from participants from the 1999–2000 and 2003–2008 National Health and Nutrition Examination Surveys (NHANES) ($n = 1766$, age 60–85 years) reported non-significant inverse associations between perfluoroalkyls and self-reported cognitive limitation consisting in difficulty remembering or periods of confusion [66]. Another cross-sectional study with 21,024 older adults (aged ≥ 50 years) who were exposed to high levels of PFOA from a chemical facility in the Mid-Ohio Valley, West Virginia, and participated in the C8 Health Project, reported statistically significant inverse associations of self-reported short-term memory loss with PFOS, PFOA, and PFHxS [67]. A study of 126 older adults aged

55–74 years and living in upper Hudson River communities which utilized various neuropsychological assessment tools (e.g., the California Verbal Learning Test, the Wechsler Memory Scale, the Wisconsin Card Sorting Test) also showed that higher PFOA and PFOS concentrations were associated with better performance in memory and learning, executive function and visuospatial function [68]. Park et al.[69], in a study on 903 adults aged ≥ 60 years from NHANES 2011–2014, found, after substantial adjustment, significant positive associations for PFOA and PFNA serum concentrations, but for PFOS a non-significant negative association, with a composite z-score for global cognition. After excluding persons suffering from chronic kidney disease, the positive associations for PFOA and PFNA were no longer significant and the negative association for PFOS became significant [69].In a study on 777 individuals aged > / = 60 from the National Health and Nutrition Examination Survey (NHANES) 2011–2014, Weng et al. [70] found that PFOA was significantly inversely associated with cognitive decline after multivariable adjustment and, in the Bayesian kernel machine regression, mixtures of 5 PFAS were significantly protective on cognitive decline in the Immediate Recall Test. Weng et al. [70] concluded that low-dose (essentially below the median value observed in their cohort) mixed PFAS was inversely associated with the risk of cognitive decline and that no significant interaction between PFAS was observed for cognitive function.

Difference in impact between adolescents and older adults

For the neurocognitive effects, it was hypothesized that the difference in impact, seen between elderly and adolescents, may have been due to the time frame difference in which they were born, implicating a higher prenatal PFAS exposure in the adolescents (born in 1995–1997) compared to the elderly group (born between 1943 and 1964). It is also likely that adverse effects on the brain might result predominantly from early exposures. The effects of endocrine disrupting agents can differ importantly in function of the time window during which exposure takes place. That is well documented [71, 72]. Mechanisms leading to adverse effects of PFAS prenatal exposure on the nervous system later in life were shown in animal experiments: Johansson et al. [73] (proteins important for neuronal growth and synaptogenesis), Lee et al. [74] (more lipid oxidation and oxidative stress in fetuses than in dams), Wang et al. [75] (reduced spatial learning and memory abilities of the offspring on postnatal day 35), Zhang et al. [76] (inhibition of long-term potentiation and changes in receptors after exposure starting in utero), and Zhang et al. [77] (tau hyperphosphorylation and beta-amyloid aggregation in adult rats after pre/postnatal PFOS exposure). Disruption of thyroid function might be involved, as thyroid hormones play an important role in the development of the brain [78]. Pedersen et al. [79] observed changes in neurochemical signaling in association with PFAS concentrations in the brain of polar bears. Additionally, exposure to PFAS mainly during later life of the adults might have induced protective effects. As proposed by Power et al. [66] and discussed by Quaak et al. [53], PFAS may have neuroprotective effects. It is known that PFAS are agonist of PPAR receptors [80]. As reviewed by Kapadia et al. [81] PPAR agonists have both neuroprotective as well as central nervous system anti-inflammatory characteristics. As PFASs are known to interfere with the immune function [11, 12, 82) and as neurodegenerative diseases are accompanied by inflammation, it may be speculated that exposure to PFASs may have a beneficial impact on brain health in older human adults. It is conceivable that increased leptin levels also play a role in possible positive cognitive effects of PFAS, especially on older people. Leptin levels were not measured in the adolescents participating in FLEHS 2 nor in adults participating in FLEHS 3. However, a weak positive association with Leptin was observed for PFAS cord plasma concentrations in mothers participating in the FLEHS 2 and FLEHS 3 campaigns, significant only for PFHxS in FLEHS 3 mothers (unpublished results of the Flemish biomonitoring, see Note on Leptin in Additional Materials). Although some animal experiments [83] and observations in humans [84, 85] found negative associations between PFAS and Leptin concentrations, often positive associations were observed. Experimental evidence for induction of higher leptin concentrations by PFAS was observed in human cells in vitro [86] and in animals [87–90] and also observations on humans showed positive associations between internal exposure to PFAS and leptin serum concentrations [91–93]. Specially relevant in relation to our findings concerning cognition in elderly people is the observation of Ding et al. [94] who found that, in women aged 45–56, higher PFAS concentrations were associated with higher leptin and free leptin values. Leptin was observed to improve memory processing in AMP8 mice, which developed elevated amyloid-beta and memory deficits with advancing age [95], and this effect was more pronounced in older AMP8 mice. Studies in transgenic mouse models of Alzheimer's disease have shown that chronic administration of leptin could ameliorate brain pathology and improve cognitive performance [96].

Strengths and limitations

As to strengths and limitations of the study, PFAS blood levels were analyzed in the same lab during the different surveys performed in a 4 year time frame. Furthermore,

well trained research nurses with much experience were responsible for the contact and interactions with the participants, including the administration of the neurobehavioral tests. Uniform study protocols facilitated comparability of the results.

Weaknesses in our study comprise that the questionnaire used to study gender identity is too simple to take into account the complexity of this issue and the societal changes involved. Many statistical associations were tested, increasing the likelihood of chance findings. However, in accordance with the views of the epidemiologist Kenneth Rothman [97], and in view of the known endocrine disrupting properties of PFAS—which render observed exposure–effect relations biologically plausible—we did not apply corrections for multiple testing. Since the studies were cross-sectional, since we could not exclude reverse causality due to reuptake of PFAS in the kidneys and through the enterohepatic circulation, this study on its own does not provide evidence for causality. However, by confirming the results of other research, it contributes to the knowledge and evidence concerning the diverse effects of PFAS.

Conclusion

Our observations point to neurobehavioral and cognitive effects of PFAS. The neurobehavioral effects might in part result from the changes in sex hormone levels that have been reported to be associated with internal exposure to PFAS. Interestingly, whereas in relation to cognition some adverse effects were recorded for adolescents, for elderly persons our observations rather suggest possible weak positive effects with respect to cognition. Our observations might be in line with the view that PFAS have many, sometimes contrasting health effects.

Abbreviations

AMGC: Department of analytical, environmental and geochemistry; AML: Algemeen Medisch Laboratorium; ATSDR: Agency for toxic substances and disease registry; BELAC: Belgische Accreditatie-Instelling; BMI: Body mass index; CPTincor: Number of incorrect reactions in the continuous performance test; CPTmean: Mean reaction time for responding to the target letter in milliseconds in the continuous performance test; DSB: Digit span backward, the maximum span (number of digits) reproduced in the in the reverse order in the digit span test; DSF: Digit span forward, the maximum span (number of digits) reproduced in the forward part of the digit span test; DSST: Digit–symbol substitution test; DSSTlat: Total time needed to complete the digit–symbol substitution test measured in seconds; DSSTnerr: Number of errors in the digit–symbol substitution test; EQAS: External quality assessment scheme; FLEHS: Flanders environment and health studies; FTT: Finger Tapping Test; FTTnpref: Total number of taps with the non-preferred hand in the finger tapping test; FTTpref: Total number taps with the preferred-hand in the finger tapping test; G-EQUAS: German external quality assessment scheme for Analyses in Biological Materials; HPLC: High-performance liquid chromatography; IQR: Interquartile; Ln-transformed: Natural logarithm has been taken, with as base the number "e"; LOD: Limit of detection; LOQ: Limit of quantification; MS/MS: Tandem mass spectrometry; NES3: Neurobehavioral evaluation system 3; OR: Odds ratio; p25: Percentile 25; p75: Percentile 75; p90: Percentile 90; PAHs: Polycyclic aromatic hydrocarbons; PBDEs: Polybrominated diphenyl ethers; PCBs: Polychlorinated biphenyls; PFAS: Poly- and perfluoroalkyl substances; PFBS: Perfluorobutane sulfonate; PFDA: Perfluorodecanoic acid; PFDoDA: Perfluorododecanoic acid; PFHxS: Perfluorohexane sulfonate; PFNA: Perfluorononanoic acid; PFOA: Perfluorooctanoic acid; PFOS: Perfluorooctane sulfonate; PFUnDA: Perfluoroundecanoic acid; PPAR-alpha: Peroxisome proliferator-activated receptor (PPAR)-alpha; PSUs: Primary sampling units; SES: Socioeconomic status; Strooper: Number of errors made in the Stroop test; Strooptime: Average time that passed between the appearance of the name and touching the correct button in the Stroop test, expressed in milliseconds; UZA: Antwerp University Hospital; VITO: Vlaams Instituut voor Technologisch Onderzoek; VUB: Vrije Universiteit Brussel.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-022-00675-3.

Additional file 1 : **Table S1.** FLEHS-2 campaign on adolescents. List of confounders and potential covariates included in stepwise multiple or logistic regressions. **Table S2.** FLESH-3 campaign on adults. List of confounders and potential covariates included in stepwise multiple or logistic regressions. **Table S3.** Association of blood concentrations of perfluoro compounds with ln-transformed cognitive and neuropsychological parameters. **Table S4.** Association of blood concentrations of perfluoro compounds with non-transformed cognitive and neuropsychological parameters.

Acknowledgements

We thank the families, mothers, adults and adolescents who participated in FLEHS 2 and 3 and the hospitals, doctors and nurses who were involved in this study. Without their effort, this study would not have been possible. We thank the field workers from the Provincial Institute of Hygiene and VITO for the sample and data collection and the "Algemeen Medisch Laboratorium" for certain measurements. The FLEHS-studies were carried out by the Flemish Center of Expertise on Environment and Health. The studies of the Center were commissioned by the Ministry of the Flemish Community. We thank in particular Karen Van Campenhout and her collaborators of the Flemish Ministry, Department of Environment, for years of constructive collaboration in the administration of the FLEHS campaigns. The views expressed herein are those of the authors and are not necessarily endorsed by the Flemish government.

Author contributions

NVL participated in the setting up of the studies, was the major contributor in writing the manuscript and was the spokesperson for the FLEHS 2 studies. GK participated in the setting up of the studies, and in the writing of the manuscript. SD performed the statistical analysis. AC participated in the setting up of the studies and in the practical organization; LB participated in the setting up of the studies and assisted in the statistical analysis. EDH participated in the setting up of the studies and in the organization of the field work. EG participated in the setting up of the studies and assisted in the statistical analysis and in the practical organization. BM participated in the setting up of the studies, in the field work and had a major role in the sociological aspects of the studies. TS performed the chemical analyses on PFAS. SR gave advice concerning the statistical analysis. DC participated in the practical organization of the studies and in the sociological aspects of the studies. TN participated in the setting up of the studies and guided the neurobehavioral and cognition aspects of the studies. VN participated in the setting up of the studies, in the organization of the field work and was the spokesperson for the FLEHS 3 studies. WB participated in the setting up of the studies and was responsible for the general coordination of part of the studies. GS participated in the setting up of the studies, was responsible for the general coordination of part of the studies and was coordinator of the field work committee. All authors read and approved the final manuscript.

Funding

This study was commissioned and financed by the Ministry of the Flemish Community (Department of Economics, Science and Innovation; Flemish Agency for Care and Health; and Department of Environment, Nature and

Energy). The sponsor was not involved in analysis and interpretation of the data, nor in the decision to publish the data.

Availability of data and materials
The aggregated data are publically available via the IPCHEM data platform. (https://ipchem.jrc.ec.europa.eu/RDSIdiscovery/ipchem/index.html#discovery). The individual records can be requested via the procedures that are available on this portal (https://ipchem.jrc.ec.europa.eu/RDSIdiscovery/ipchem/index.html#showmetadata/FLEHS1REFNB).

Declarations

Ethics approval and consent to participate
In order to participate all subjects had to give written informed consent. The FLEHS studies were approved by the Ethics Committees of the University of Antwerp and the Antwerp University Hospital (UZA), Belgium. The dossier numbers for the different studies were, respectively, UA A08 09 (FLEHS-2, neonates, adults and adolescents of industrial contaminated site) and B300201419843 for FLEHS-3 adults. All methods were carried out in accordance with relevant guidelines and regulations (Declaration of Helsinki).

Consent for publication
Not applicable.

Competing interests
"The authors declare that they have no competing interests".

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Received: 30 March 2022 Accepted: 17 July 2022
Published online: 30 September 2022

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