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Association between dietary intake estimated levels of PCDD/Fs and human sperm quality

María Ángeles Martínez [a,b,c,g,1], Albert Salas-Huetos [a,c,d,e,*], María Fernández de la Puente [a,b,c], Cristina Valle-Hita [a,b,c], Nadine Khoury [a,b,c], Elena Sánchez-Resino [a,b,f], Carla Ramos-Rodríguez [a,b,d], Estefanía Davila-Cordova [a,b], Jordi Salas-Salvadó [a,b,c,2], Nancy Babio [a,b,c,2]

[a] Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain
[b] Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Alimentaciò, Nutrició, Desenvolupament i Salut Mental ANUT-DSM, Reus, Spain
[c] Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Institute of Health Carlos III, Madrid, Spain
[d] Unit of Preventive Medicine and Biostatistics, Faculty of Medicine and Health Sciences, Universitat Rovira i Virgili, Reus43201 Spain
[e] Department of Nutrition, Harvard T.H. Chan School of Public Health, Harvard University, Boston, MA US-02115, USA
[f] Universitat Rovira i Virgili, Laboratory of Toxicology and Environmental Health, School of Medicine, Sant Llorenç 21, Reus, Catalonia 43201, Spain
[g] Department of Pharmacology, therapeutics and Toxicology, Faculty of veterinary. Universitat Autònoma de Barcelona, Cerdanyola del Vallès 08193, Catalonia, Spain

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ABSTRACT

This study aimed to investigate the association between estimated dietary intake of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and human sperm quality. This study cross-sectionally assessed the associations between estimated dietary intake of PCDD/Fs and sperm quality parameters in 200 participants aged 18–40 years from the Led-Fertyl study. Linear regression models, accounting for potential confounding variables, were employed to evaluate the relationships. To estimate the PCDD/Fs exposure, food frequency questionnaires and the latest data on PCDD/Fs concentrations in food, primarily from Spanish sources, were used. Our findings indicate that, in comparison to participants in the lowest tertile, those in the highest tertile (T3) of PCDD/Fs dietary intake exhibited significantly elevated body mass index, increased consumption of meat, fish and eggs, and decreased consumption of nuts. Furthermore, individuals in T3 demonstrated a higher percentage of sperm head abnormalities (4.65 % [0.10; 9.24]; p-trend= 0.037) and a corresponding increase per 1-SD increment in energy-adjusted total PCDD/Fs dietary intake (1.84 % [0.38; 3.68]). No significant associations for other sperm parameters were found. Minimal research exists on PCDD/F dietary exposure and human sperm quality. This study shows significant direct association between higher PCDD/Fs intake and the percentage of sperm head abnormalities which potentially may compromise human reproductive health.

1. Introduction

The prevalence of infertility has markedly risen over the past decade, impacting approximately 12–15 % of the global population, with male factors contributing to nearly 50 % of these cases [1,2]

It is well established that infertility is a disease that can occur due to the interaction of several factors, such as genetic predisposition and other parameters related to the exposome such as diet [1], physical

Abbreviations: BMI, Body mass index; DI, Dietary intake; EDCs, Endocrine disrupting chemicals; EFSA, European Food Safety Authority; FFQs, food frequency questionnaires; PCDD/Fs, polychlorinated dibenzo-p-furans; POPs, persistent organic pollutants; TEQ, Toxic Equivalents; TWI, Tolerable weekly intake.

* Correspondence to: Unit of Preventive Medicine and Biostatistics, Departament de Bioquímica i Biotecnologia, Alimentaciò, Nutrició, Desenvolupament i Salut Mental ANUT-DSM Faculty of Medicine and Health Sciences, Universitat Rovira i Virgili, Reus ES-43201, Spain

E-mail address: albert.salas@urv.cat (A. Salas-Huetos).

[1] Present address: Department of Pharmacology, therapeutics and Toxicology, Faculty of veterinary. Universitat Autònoma de Barcelona, Cerdanyola del Vallès 08193, Catalonia, Spain

[2] These authors should be listed as senior-authors

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activity, and environmental exposures among others [3,4]. Specifically, regarding the environmental factors, the exposure to a family of chemical compounds in our daily life, recognized as endocrine disrupting chemicals (EDCs) could be seriously implicated [5] Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are enduring environmental contaminants, categorized as persistent organic pollutants (POPs), persisting in the environment and accumulating within the food chain. These chemicals are recognized as EDCs, with long half-lives and with the capacity to mimic or block the action of endogenous hormones, leading to adverse effects on male reproductive health [5,6]. PCDD/Fs mainly reach humans through dietary sources and they can bioaccumulate in the human body due to their lipophilic properties, high metabolic stability, and resistance to degradation [7,8]. Moreover, these substances are acknowledged as obesogenic factors [9], and there is a growing body of evidence indicating potential connections between exposure to these EDCs and prevalent chronic conditions such as type 2 diabetes [10], metabolic syndrome [11], and cardiovascular diseases [12]. However, epidemiological evidence linking PCDD/Fs to sperm quality or male infertility is currently limited and heterogeneous [13].

PCDD/Fs have diverse origins, including natural occurrences and unintended by-products generated in various thermal and industrial processes. It is essential to clarify that the presence of PCDD/Fs in food products is primarily due to environmental contamination and unintentional incorporation during manufacturing. Consequently, exposure to PCDD/Fs is widespread, raising significant concerns about potential adverse effects on human health [14].

In-depth exploration of the impact of dioxin exposure on male reproductive function has been more extensively studied in animals than in humans [15]. Literature data on human studies are scarce; in a recent systematic review and meta-analysis, diverse endocrine disruptors were thoroughly examined, including the dioxin family, in biological matrices and sperm quality parameters. However, the overall results revealed a large heterogeneity regarding the relationship between these EDCs and sperm quality [13].

Dietary sources such as fish, red meat, and dairy products predominantly contribute to PCDD/Fs exposure, comprising over 90 % of the total exposure [16]. Therefore, the current study hypothesizes that dietary exposure to PCDD/Fs, recognized as EDCs, would negatively impact semen quality parameters. Consequently, the aim was to conduct a cross-sectional examination to evaluate the correlations between the estimated dietary intake of PCDD/Fs and sperm quality parameters among 200 participants aged 18–40 years from the Led-Fertyl cohort study population. These findings offer novel insights, paving the way for additional evidence and informing future public health strategies.

2. Materials and methods

2.1. Research design and characteristics of the study population

The investigation employed a cross-sectional design, drawing upon data from a cohort of 200 healthy men enrolled in the Led-Fertyl "*Lifestyle and Environmental Determinants of Seminogram and other Male Fertility-related Parameters*" study (Fig. 1). Eligibility criteria for participants included: healthy volunteers aged between 18 and 40 years, drawn from the general population. The inclusion and exclusion criteria are full described in Supplementary Material**, Annex 1**. Participants were recruited between February 2021 and April 2023, and lifestyle data, sociodemographic characteristics, medical data and dietary habits were collected by on-line questionnaires. Subsequent to recruitment, participants underwent assessments at the Hospital Universitari Sant Joan de Reus (Reus, Tarragona, Spain), involving measurements of anthropometric parameters and the collection of biological samples, including urine, blood, and semen. All participants provided both online and written informed consent, and the project protocol received approval from the Ethical Committee of Institut d'Investigacions Sanitàries Pere i Virgili (Ref. CEIM: 181/2019).

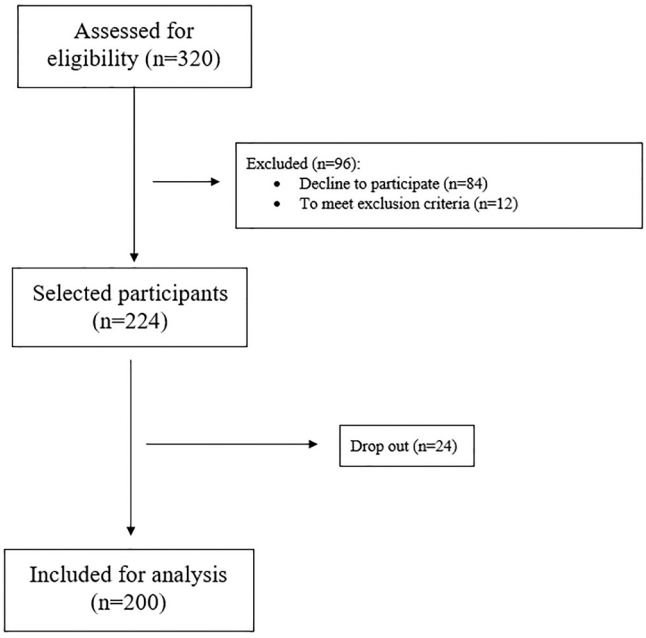

Fig. 1. Flow chart.

2.2. Dietary intake assessment

Validated semiquantitative food frequency questionnaires (FFQ) [17] were administrated to the participants of all the study cohort. They documented their average consumption patterns, indicating both frequency and quantity for 143 food and beverage items over the preceding year. The FFQ were developed in an optically readable format and they were scanned. Consequently, e-Diet Base URV software [18] automatically exports the frequency and amount of consumption in g/day based on the Spanish food composition database [19]. The concentration levels of the PCDD/Fs expressed in terms of Toxic Equivalents (TEQ) were used to perform the calculations of dietary intake [20].

The analysis incorporated the latest data on PCDD/Fs in food, with a primary focus on studies that assessed the levels of these contaminants in food from the Mediterranean region. These detailed data can be found elsewhere [9]. Briefly, Data were presented as picograms of Toxic Equivalent (pg TEQ) per gram of fresh weight. Coffee and alcoholic beverages were omitted from the dietary assessment, given their typically negligible levels of PCDD/Fs, contributing minimally to the overall dietary intake evaluation [9]. Consequently, PCDD/Fs dietary intake (DI) was estimated following this equation previously used by Khoury et al., 2023 [9]:

$$DI_{\text{PCDD/Fs (pgTEQ /week)}} = (\sum TEQ_i \cdot A_i) \cdot 7$$

$DI_{PCDD/Fs}$ *is the PCDD/Fs dietary intake; TEQi signifies the toxic equivalency of a specific food item (in pg PCDD/Fs/g), and Ai represents the quantity of a particular food consumed (in g/day). Multiplying this by 7 yielded the* $DI_{PCDD/Fs}$ *in picograms TEQ per week.*

Regarding the use of data from Khoury et al. (2023), although the dataset includes data from other countries, we would like to highlight that nearly 90 % of the data are from Spain. These Spanish data are from 2018 and represent the most comprehensive and up-to-date source of information on chemical content in a wide variety of food items [21]

The dietary intake of PCDD/Fs (expressed in TEQ pg/week) was adjusted for total energy intake using the residual-regression method by Willett and Stampfer, 1986 [22] and categorized in tertiles of energy-adjusted (TEQ pg/week) corresponding to lower (first tertile), intermediate (second tertile), and higher (third tertile) dietary intake of PCDD/Fs. This method involves regressing the dietary intake of

PCDD/Fs against total energy intake to obtain residuals, which represent PCDD/Fs intake independent of energy consumption.

The European Food Safety Authority (EFSA) has set the tolerable weekly intake for PCDD/Fs at 2.0 pg TEQ/kg of body weight per week (TEQ/kg_{bw}/week) [23]. Therefore, to calculate exposure in our study population, body weight was incorporated as the dividend in the equation described previously expressed as TEQ/kg_{bw}/week. This allowed us to determine whether participants in the Led-Fertyl study were above or below the EFSA cut-off values.

2.3. Semen analysis

Semen samples were obtained by masturbation after a minimum of 3 days of sexual abstinence. The participants were instructed to collect the sample in a sterile standard-polypropylene container. Sperm quality parameters were analysed after liquefaction (30 min at 37ºC). Macroscopic parameters included semen volume and pH. Microscopic parameters were examined through the utilization of a phase-contrast microscope and a computer-assisted sperm analysis (CASA) system (SCA, Microptic). This analysis encompassed key factors such as sperm count and concentration, sperm motility, sperm vitality, and sperm morphology. Collection and examination of semen samples followed the World Health Organization 2010 standards [24]. Sperm count and concentration were measured at × 400 magnification and expressed as millions of spermatozoa per ejaculate or millions of sperm cells per ml, respectively. Sperm motility was assessed by evaluating different photos taken by the CASA system and every sperm cell was subsequently classified as: progressive motile, nonprogressive motile or immotile. Motility was further quantified as a percentage of the overall total motility. Sperm vitality was assessed through the hypoosmotic swelling test (HOS test) at × 400 magnification, analysing 200 sperm cells. Additionally, sperm morphology was examined using the Hemacolor (Millipore) staining protocol, observing 200 sperm cells under × 1000 brightfield optics with immersion oil. Morphology was assessed by quantifying the percentage of normal forms or the percentage of abnormalities in the head, midpiece, or terminal piece.

2.4. Statistical analyses

Ledfertyl is an ongoing cohort study, the most recent version of the Led-Fertyl database (updated in May 2023), containing data from 200 participants, was used for the statistical analysis. Covariates were assessed using prior knowledge and descriptive statistics from our cohort study population. Table 1 presents continuous variables as mean ± SD or medians [25th-75th percentiles], depending on their distribution (normal or non-normal). Categorical variables are shown as percentages (number). Baseline characteristics were compared across PCDD/Fs energy-adjusted tertiles using one-way ANOVA for continuous variables and the Chi-square test for categorical variables.

To evaluate the association, multiple linear regression models were employed, providing the β-coefficient (95 % confidence interval (CI)) for sperm parameters concerning tertiles of energy-adjusted total dietary of PCDD/Fs intake and expressed as 1-SD increase in energy-adjusted total PCDD/Fs dietary intake. Three regression models were considered, adjusted for several a priori selected potential confounders [25–27]: a) crude model; b) Model 1, adjusted for age (years); and c) Model 2, where adjustments were made for age, marital status (categorized as single, married, or other), education level (grouped into up to high school, college or higher education), smoking habits (categorized as never, current, or former), body mass index (BMI) in kg/m^2, physical activity measured in METs·min/week, sexual abstinence period (days preceding semen sample delivery), and the validated 14-item Mediterranean Diet Adherence Screener (MEDAS) [28]. In the regression analyses, semen volume, sperm concentration, sperm total count, normal forms, abnormal midpiece, and abnormal terminal piece underwent a square root transformation to better align with the normality

Table 1
Characteristics of included participants overall and across energy-adjusted total PCDD/Fs dietary intake tertiles.

	Energy-adjusted total PCDD/Fs dietary intake (TEQ pg/week)				
	All	**T1**	**T2**	**T3**	***p*-value**
	n = 200	n = 67	n = 67	n = 66	
	244 ± 90.7	180 ± 63.8	234 ± 49.2	317 ± 94.2	
Age (years)	28.4 ± 5.53	28.0 ± 5.70	29.2 ± 6.20	28.2 ± 4.80	0.404
Educational level	35.5 (71)	34.3	40.3	31.8	0.576
Up to high school	64.5	(23)	(27)	(21)	
College or high	(129)	65.7	59.7	68.2	
education		(44)	(40)	(45)	
Marital status	88.5	91.0	83.6	90.9	0.474
Single	(177)	(61)	(56)	(60)	
Married	10.0 (20)	7.5 (5)	13.5 (9)	9.0 (6)	
Other	1.50 (3)	1.5 (1)	3.0 (2)	0 (0)	
Monthly income	15.50	16.42	13.4	16.7	0.483
(Euros)	(31)	(11)	(46)	(11)	
< 1000	65.0	62.7	73.1	59.1	
1000–2000	(130)	(42)	(16)	(39)	
> 2000	19.5 (39)	20.9	13.4 (2)	24.2	
		(14)		(16)	
Smoking status	72.9	71.8	76.6	73.0	0.999
Never	(137)	(46)	(45)	(46)	
Current	13.3 (25)	14.1 (9)	13.1 (8)	12.7 (8)	
Former	13.8 (26)	14.1 (9)	13.1 (8)	14.3 (9)	
Physical activity	4086	4395	3505	4364	0.168
(MET min/week)	± 3093	± 2926	± 2637	± 3606	
Waist	83.2	81.5	83.8	84.0	0.124
circumference (cm)	± 8.35	± 8.00	± 9.17	± 7.78	
BMI (kg/m^2)	24.4	23.6	24.5	25.1	**0.018**
	± 3.05	± 3.10	± 3.34	± 3.05	
Adherence to	8.02	8.03	7.60	8.45	**0.029**
Mediterranean diet	± 1.86	± 1.92	± 1.77	± 1.84	
MEDAS score (0–14 points)					
Dietary assessment	2647	2715	2539	2687	0.225
Energy intake (kcal/	± 633	± 673	± 545	± 669	**< 0.001**
day)	107	98.8	103	120	**< 0.001**
Proteins (g/d)	± 29.6	± 28.5	± 24.0	± 32.2	0.446
Proteins (% in kcal	16.2	14.6	16.3	17.9	**0.046**
from total intake)	± 2.42	± 2.03	± 1.78	± 2.18	**0.010**
Total fat (g/d)	125	124	121	129	**< 0.001**
Total fat (% in kcal	± 33.9	± 39.7	± 29.1	± 31.8	**0.011**
from total intake)	42.5	41.0	43.2	43.4	0.302
Carbohydrates (g/d)	± 6.11	± 6.68	± 6.31	± 5.00	0.078
Total carbohydrates	258	283	245	248	0.152
(% in kcal from total	± 80.3	± 84.7	± 73.6	± 77.8	0.152
intake)	38.8	41.6	38.3	36.5	**< 0.001**
Dietary fiber (g/d)	± 6.54	± 7.08	± 6.28	± 5.11	**< 0.001**
Fruits and vegetables	24.9	28.1	23.0	23.6	**< 0.001**
(g/d)	± 10.9	± 14.0	± 8.77	± 8.32	**< 0.001**
Legumes (g/d)	521	541	485	537	0.841
Total cereals (g/d)	± 233	± 255	± 192	± 245	**0.021**
Milk and dairy	28.1	32.8	25.7	25.7	0.579
products (g/d)	± 21.0	± 24.9	± 19.1	± 17.6	0.335
Eggs (g/d)	151	165	148	139	0.218
Red meat and	± 78.3	± 85.2	± 82.4	± 64.6	
derivatives (g/d)	283	265	264	320	
White meat (g/d)	± 193	± 212	± 144	± 212	
Fish and seafood (g/	43.3	32.9	39.0	58.3	
d)	± 39.5	± 26.9	± 33.6	± 50.4	
Oils and fats (g/d)	91.9	66.1	96.7	113	
Nuts (g/d)	± 59.5	± 58.0	± 49.1	± 61.6	
Ready to eat (g/d)	67.1	43.7	71.5	86.2	
Juices (g/d)	± 40.4	± 33.9	± 25.4	± 47.0	
Biscuits and pastries	85.1	66.5	78.3	110	
(g/d)	± 46.2	± 44.7	± 37.7	± 47.2	
	36.8	37.4	37.3	35.9	
	± 16.4	± 18.8	± 15.2	± 14.9	
	22.2	28.1	21.2	17.2	
	± 23.0	± 30.3	± 20.4	± 14.2	
	45.7	49.1	44.4	43.6	
	± 32.8	± 34.2	± 31.8	± 32.5	

(*continued on next page*)

Table 1 (*continued*)

	Energy-adjusted total PCDD/Fs dietary intake (TEQ pg/week)				
	All n = 200 244 ± 90.7	**T1** n = 67 180 ± 63.8	**T2** n = 67 234 ± 49.2	**T3** n = 66 317 ± 94.2	***p*-value**
	45.7 ± 71.6	52.3 ± 73.0	35.3 ± 48.9	49.6 ± 87.5	
	41.0 ± 38.7	47.7 ± 41.3	36.6 ± 30.8	38.8 ± 42.9	
Semen volume (ml)	3.5 [2.5; 4.5]	3.5 [2.5; 4.4]	3.5 [2.7; 5.0]	3.3 [2.2; 4.5]	0.619
Total sperm count ($\times 10^6$)	163 [94.5; 284]	183 [117; 288]	144 [75; 292]	143 [101; 258]	0.569
Sperm concentration ($\times 10^6$/ml)	48.4 [28.7; 83.4]	49.7 [32.8; 81.7]	45.9 [21.6; 86]	49.8 [28.5; 82.1]	0.937
Total motility (%)	59.6 ± 17.4	60.3 ± 17.2	55.9 ± 19.5	62.4 ± 15.2	0.089
Immotile spermatozoa (%)	39.1 ± 16.7	38.3 ± 16.1	42.0 ± 18.8	37.1 ± 14.7	0.189
Normal forms (%)	9.0 [5.0; 15]	7.5 [4.5; 16.5]	9.5 [4.5; 14.5]	10 [5.5; 15]	0.776
Abnormal head (%)	34.2 ± 12.8	31.5 ± 12.2	35.9 ± 13.7	35.2 ± 13.4	0.097
Abnormal midpiece (%)	2.0 [0.5; 3.0]	1.5 [0.5; 2.5]	1.9 [0.5; 3.5]	2.0 [1.0; 3.0]	0.458
Abnormal terminal piece (%)	4.0 [2.0;8.0]	5.5 [2.0; 10.5]	3.0 [1.5; 6.0]	4.5 [2.0; 7.5]	0.109

Abbreviations: PCCD/Fs: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; BMI: body mass index. Continuous variables are expressed as mean ± SD, or medians [25th-75th percentiles] and categorical variables are presented as percentages (number). *p*-values for comparisons were tested by one-way ANOVA or Chi-square test, as appropriate across tertiles.

assumption of the residuals.

Additionally, sensitivity analyses considering the exposure to PCDD/Fs (in pgTEQ/kg_{BW}/week) categorically, above and below the recommendation values established by EFSA, and its association with sperm quality parameters were executed to verify the robustness of the results.

The statistical analysis was carried out using Stata 14 (StataCorp) software, and a significance level of $p < 0.05$ was established for the results.

3. Results

In the present analysis, we included 200 healthy men (mean age: 28 ± 5.53). The baseline characteristics of the subjects across energy-adjusted total PCDD/Fs dietary intake tertiles (TEQ pg/week) are depicted in Table 1. Our findings revealed that participants in the highest tertile of PCDD/Fs dietary intake (T3) exhibited significantly elevated BMI, along with increased consumption of fish, meat, and eggs, and notably lower intake of nuts. Fig. 2 illustrates the percentage contribution of each food item to the total dietary intake of PCDD/Fs, and the total dietary exposure of PCDD/Fs (in pgTEQ/kg_{bw}/week). Red meat (30 %), followed by fish and seafood (16 %), and white meat (14 %) emerged as the primary contributors to the overall dietary exposure. Additionally, those in the highest tertile of energy-adjusted dietary PCDD/Fs intake demonstrated higher protein consumption and lower intake of carbohydrates and dietary fiber. These findings align with prior published articles that have consistently highlighted the significant role of animal-based foods, particularly meat and fish items, as the primary contributors to the overall intake of PCDD/Fs [29–31].

Table 2 presents multivariable-adjusted β-coefficients and their corresponding 95 % confidence intervals for seminogram parameters across tertiles of estimated PCDD/Fs dietary intake, as well as expressed as 1-SD increment of total PCDD/Fs dietary intake. Elevated dietary intake of PCDD/Fs (T3) exhibited a statistically significant association with a higher percentage of abnormal spermatozoa head: ((β coefficient [95 %CI]) (4.65 % [0.10–9.24])) and *p* for trend = 0.037 for the full-adjusted model and ((β coefficient [95 %CI]) (1.84 % [0.38–3.68])) (per 1-SD increment). No other significant associations were found for the rest of sperm parameters. Although non-significant, the trend was in the expected direction for semen volume, total sperm count, and sperm concentration, and the percentage of abnormal midpiece.

In our study, the mean exposure levels to PCDD/Fs in each tertile (T) were as follows: T1 (n = 67): 180 ± 63.8 TEQ pg/week; T2 (n = 67): 234 ± 49.2 TEQ pg/week and T3 (n = 66): 317 ± 94.2 TEQ pg/week, being the overall Mean: 244 ± 90.7 TEQ pg/week. Consequently, in our study, 88 % of the participants were above the cut-off value established by the EFSA for PCDD/Fs, with a mean and P5; P95 (5th; 95th percentile) of 3.13 (1.53; 5.13) pg/kgbody weight/week. In order to validate the robustness of the results, sensitivity analyses were carried out, determining multivariable-adjusted β-coefficients and their corresponding 95 % confidence intervals for seminogram parameters in

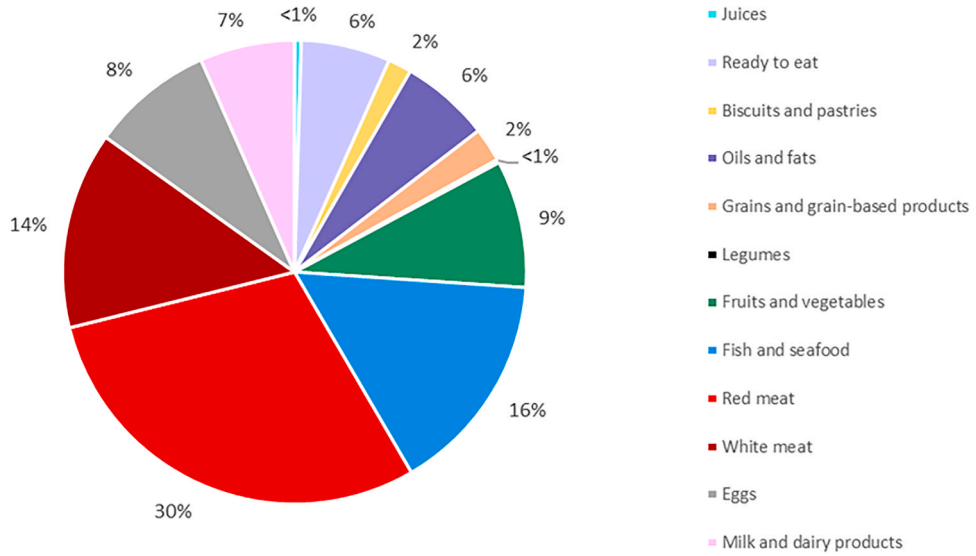

Fig. 2. Contribution of the food items (%) from the total dietary intake of PCDD/Fs. Mean and (5th; 95th percentile): 3.13 (1.53; 5.13) pg/$kg_{body\ weight}$/week).

Table 2
Adjusted β-coefficients and their corresponding 95 % confidence were calculated for seminogram parameters across tertiles of energy-adjusted total PCDD/Fs dietary intake tertiles as well as per 1-SD increment of energy-adjusted total PCDD/Fs dietary intake.

	Energy-adjusted total PCDD/Fs dietary intake (TEQ pg/week)				
	Tertile 1 n = 67	**Tertile 2** n = 67	**Tertile 3** n = 66	***p*-trend**	**Continuous (1 SD*)**
Energy adjusted total PCDD/Fs dietary intake (TEQ pg/week), mean ± SD	180 ± 63.8	234 ± 49.2	317 ± 94.2		
β-coefficients (95 %CI)					
Sperm characteristics					
Semen volume[a] (ml)	**0 (Ref.)**	0.02 (-0.11 to 0.15)	-0.04 (-0.17 to 0.10)	0.535	-0.00 (-0.05 to 0.49)
Crude model	**0 (Ref.)**	0.02 (-0.11 to 0.14)	-0.04 (-0.17 to 0.10)	0.533	-0.00 (-0.05 to 0.49)
Model 1	**0 (Ref.)**	0.04 (-0.09 to 0.17)	-0.04 (-0.17 to0.90)	0.576	0.00 (-0.05 to 0.49)
Model 2					-0.00 (-0.54 to 0.52)
Total sperm count[a] ($\times 10^6$)	**0 (Ref.)**	-1.10 (-3.10 to 0.87)	-0.82 (-2.80 to 1.17)	0.403	-0.43 (-1.24 to 0.37)
Crude model	**0 (Ref.)**	1.27 (-3.24 to 0.69)	0.82 (-2.78 to 1.15)	0.395	-0.42 (-1.22 to0.38)
Model 1	**0 (Ref.)**	1.11 (-3.05 to 0.84)	1.02 (-2.98 to 0.95)	0.293	-0.53 (-1.33 to0.26)
Model 2					
Sperm concentration[a] ($\times 10^6$/ml)	**0 (Ref.)**	-0.36 (-1.42 to 0.69)	-0.06 (-1.12 to 1.00)	0.890	-0.17 (-0.61 to 0.25)
Crude model	**0 (Ref.)**	0.42 (-1.48 to 0.64)	0.06 (-1.12 to 1.00)	0.886	-0.16 (-0.60 to 0.26)
Model 1	**0 (Ref.)**	0.38 (-1.45 to 0.68)	0.13 (-1.20 to 0.94)	0.785	-0.21 (-0.63 to 0.22)
Model 2					
Total motility (%)	**0 (Ref.)**	-4.44 (-10.34 to 1.46)	2.06 (-3.86 to 7.99)	0.545	-0.11 (-2.56 to2.33)
Crude model	**0 (Ref.)**	4.06 (-9.96 to 1.84)	2.06 (-3.84 to 7.97)	0.539	-0.15 (-2.59 to2.27)
Model 1	**0 (Ref.)**	4.46 (-9.79 to 2.14)	1.90 (-3.90 to 7.90)	0.598	-0.37 (-2.84 to 2.09)
Model 2					
Immotile spermatozoa **(%)**	**0 (Ref.)**	3.97 (-1.70 to 9.64)	-1.02 (-6.72 to 4.66)	0.771	0.33 (-2.01 to 2.67)
Crude model	**0 (Ref.)**	3.70 (-1.98 to 9.39)	-1.02 (-6.72 to 4.66)	0.768	-0.36 (-1.97 to 2.70)
Model 1	**0 (Ref.)**	2.63 (-3.13 to 8.38)	-1.77 (-7.58 to 4.04)	0.594	-0.15 (-2.57 to 2.37)
Model 2					
Normal form[a] **(%)**	**0 (Ref.)**	0.04 (-0.36 to 0.45)	0.18 (-3.62 to 0.45)	0.369	0.08 (-0.09 to0.25)
Crude model	**0 (Ref.)**	0.05 (-0.33 to 0.47)	0.18 (-0.21 to 0.59)	0.370	0.08 (-0.08 to1.24)
Model 1	**0 (Ref.)**	0.81 (-0.33 to 0.42)	0.21 (-0.20 to 0.62)	0.318	0.09 (-0.07 to0.74)
Model 2					
Abnormal head **(%)**	**0 (Ref.)**	**4.90 (0.54 to 9.25)**	3.28 (-1.07 to 7.64)	0.120	1.70 (-0.10 to 3.46)
Crude model	**0 (Ref.)**	**4.93 (0.54 to 9.25)**	3.27 (-1.08 to 7.64)	**0.037**	1.67 (-0.11 to 3.46)
Model 1		**4.88**	**4.65**		**1.84 (0.38 to 3.68)****
Model 2					
Abnormal midpiece[a] **(%)**	**0 (Ref.)**	0.12 (-0.13 to 0.37)	0.17 (-0.08 to 0.42)	0.180	0.07 (-0.32 to 0.17)
Crude model	**0 (Ref.)**	0.11 (-0.13 to 0.36)	0.17 (-0.08 to 0.42)	0.181	0.07 (-0.32 to 0.17)
Model 1		0.13 (-0.12 to 0.38)	0.22 (-0.03 to 0.47)	0.086	0.08 (-0.13 to0.19)
Model 2					
Abnormal terminal piece[a] **(%)**	**0 (Ref.)**	**-0.46 (-0.85 to -0.69)**	-0.17 (-0.56 to 0.22)	0.336	-0.08 (-0.24 to 0.73)
Crude model	**0 (Ref.)**	**-0.44 (-0.84 to -0.53)**	0.17 (-0.56 to 0.22)	0.336	-0.09 (-0.25 to 0.71)
Model 1		**-0.44 (-0.84 to -0.04)**	0.25 (-0.65 to 0.15)	0.185	0.11 (-0.27 to0.05)
Model 2					

Abbreviations: PCCD/Fs: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; SD: Standard deviation.
Multivariable linear regression models were used to assess the β-coefficients, and its 95 % CI for sperm parameters across tertiles of energy-adjusted total dietary PCDD/Fs intake and per 1-SD increment of energy-adjusted total PCDD/Fs dietary intake. Crude model: unadjusted for covariates; Model 1: adjusted for age. Model 2: Model 1 additionally adjusted for sexual abstinence (days before delivery of the semen sample), physical activity (MET min/week), body mass index (kg/m^2), marital status (single, married, other), smoking status (never, current, former), education level (up to high school, college or high education), and adherence to Mediterranean diet MEDAS score (0–14 points).
*One SD = 72.22 TEQ pg/week
** *p*-value < 0.05
[a] Semen volume, sperm concentration, sperm total count, normal form, abnormal midpiece and abnormal terminal piece were transformed by square root to approach normality of the residuals.

accordance with EFSA recommendations on PCDD/Fs exposure, distinguishing between values above and below the reference TWI thresholds. Although no significant associations were found in this sensitivity analysis, the following sperm parameters were in the same direction than in the primary analysis: semen volume, total sperm count, sperm concentration, abnormal head, and abnormal midpiece (Supplementary Material, **Annex 2**).

4. Discussion

To our knowledge, this study is pioneering in its revelation of an association between heightened dietary intake of PCDD/Fs and sperm head abnormalities, as well as its correlation with BMI.Most of the previous studies utilized fasting blood samples for the PCDD/Fs exposure assessment. This difference in assessment methods makes direct comparison with existing literature challenging. Although, previous studies in humans have shown an association between dioxins and dioxin-like compounds in biological matrices and poor sperm quality parameters in general population, the available data are limited and heterogeneous [13]. Nevertheless, our findings relating these chemicals with sperm head abnormalities align with the potential negative impact of PCDD/Fs on sperm found in other published articles considering biomonitoring assessment. For example, the Mocarelli et al., 2008 study (n = 135) found an inverse association between 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) with sperm concentration and total count [27], while the Paul et al., 2017 study (n = 56) demonstrated a negative correlation between dioxin-like PCBs (including PCB congeners 77, 81, 123, 126, 169, 118 and 189) with semen volume [32]. In

contrast, more recently, the Petersen et al., 2018 study (n = 263) investigated dioxin-like PCBs (including PCB 105, PCB 118, and PCB 156) and their association with semen quality, revealing a non-significant correlation with sperm quality [33]. However, discrepancies in the biological matrices used to measure these disruptors (primarily in plasma or serum, and less frequently in other biological matrices), as well as variations in total chemical exposure across different populations, make it difficult to compare studies. Furthermore, it is important to note that two out of these three studies had relatively small sample sizes (<200 participants). For all these reasons, it is crucial to employ standardized methods for exposure assessment and sperm parameter determination to enable meaningful comparisons across studies. Therefore, gold standard methods of exposure assessment and sperm parameters determinations should be applied to be able to compare between studies, as information in this field is still limited. In addition, more studies with larger samples of participants in the general population is needed to reduce the current gaps in knowledge on this important topic. It points out the need of expanding our knowledge on these EDCs and to better understand all the possible causes of the current increase in infertility as well as other chronic related diseases including metabolic disorders, obesity and diabetes [34].

Nevertheless, the consistency of our results with previous studies on sperm quality, particularly in relation to head abnormalities, reinforces the hypothesis that sperm structure is particularly vulnerable to dioxin exposure.

In animals, the effects of PCDD/Fs exposure on male fertility have also been explored. These POPs have been related with negative effects on sperm parameters. In rats, chronic exposure has demonstrated a dose-dependent decrease in fertility and increased embryotoxicity [23]. However, research on the acute effects of these disrupting chemicals on human semen parameters and fertility is currently limited [35]. Moreover, both animal and *in vitro* studies have demonstrated that PCDD/Fs may exert potential effects on adiposity and contribute to metabolic disorders [34,36,37], which are also recognized factors related to infertility.

It is important to note that the present findings indicate a significant association between higher estimated dietary intake of PCDD/Fs and an increase in abnormal head morphology. It is largely known that this defect may affect the capacity of the sperm to penetrate the egg [38,39], and even if it is fertilized, these eggs may grow abnormally for a few divisions and then stop, or in the worst-case scenario, if it is transferred they may grow to the point of implanting prematurely in the uterine wall and then causing a miscarriage [40–42]. In addition, there is growing evidence showing that the exposure to POPs, especially during crucial periods of life such as early development and reproductive age, is of great concern, as this harmful effect would not only affect the individual and might be transferred to the next generation through germ cells, consequently it is vital to identify and tackle all potential contributing factors. [43–45].

The EFSA has set the current tolerable weekly intake (TWI) for PCDD/Fs at 2.0 pgTEQ/kg of body weight per week [23]. Consequently, in our study, the 88 % of the participants were above this cut-off value with a mean and P5; P95 (5th; 95th percentile) of 3.13 (1.53; 5.13) pg/kgbody weight/week. This mean dietary intake is similar to the mean reported by other Spanish and European countries [9,14,21, 46–48]. In last years a reduction in the release of PCDD/Fs and dioxin-like substances into the environment and in human exposure has been observed probably because a comprehensive control of industrial emission sources and the establishment of a number of strategies aimed at reducing the presence of these substances in food and feed [23]. Given that food consumption represents the primary pathway for human exposure to PCDD/Fs, it is imperative to implement measures aimed at reducing the levels across the entire food chain [21]. This is important especially for those food group that represent the largest exposure. In our population red meat (30 %), followed by fish and seafood (16 %), and white meat (14 %) emerged as the primary contributors to the overall dietary exposure. Therefore, efforts are still needed to not exceed the reference levels established by authorities. Furthermore, it is crucial to note that TWI reference values provided by EFSA are based on data from 2018, and perhaps a re-evaluation with more data available would be necessary to establish new maximum tolerable exposure levels.

The present study has limitations and strengths. Firstly, given the inherent nature of observational studies, it's important to acknowledge the potential for reverse causality and residual confounding from unassessed factors in our analyses. Consequently, establishing a cause-and-effect relationship becomes challenging. Secondly, the calculation of exposure through food using the FFQs as a tool could be considered a limitation, since despite the questionnaire used has been validated in Spain, all FFQs are prone to dietary measurement errors. As mentioned before, to the best of our knowledge, this study is the only one comparing estimated dietary exposure to PCDD/Fs with sperm quality parameters. Most of the previous studies [27,33] discussed utilize fasting blood samples for exposure assessment. This difference in assessment methods makes direct comparison with existing literature challenging. Therefore, assessing the exposure to these chemicals through biomonitoring in biological samples may decrease the aforementioned measurement errors. However, it is important to mention that > 90 % of human exposure to these EDCs comes from diet and therefore this deterministic assessment technique could be considered as a non-invasive and cheaper technique. These estimates may not fully capture the current exposure levels or reflect individual variations in body burdens. Nevertheless, this methodology is increasingly employed in epidemiological research because it serves as an initial screening tool to assess population-level exposure. This allows for the identification of high-risk groups or exposure trends, which can then be followed up with more detailed biomonitoring studies to provide a more precise understanding of individual exposure levels and associated health risks. Lastly, the observed results in a healthy population of Spanish individuals may not be extended to other populations with different level of exposure to these chemicals. As strength, it should be emphasized that this is one of the few articles collecting data from a quite large healthy population compared to previously published studies, providing more robust data for the analysis.

Although these findings support an association between PCDD/Fs intake and sperm quality parameters, a wider evaluation of these relationships should be explored in the future using large prospective study cohorts, biomonitoring peripheral levels of PCDD/Fs in addition to estimate dietary exposures, and measuring other potential fertility biomarkers (e.g., sperm DNA fragmentation, sperm methylation, etc.). The research question, even though seems partially answered, deserves additional attention.

5. Conclusions

In conclusion, the findings indicate an association between increased estimated dietary intake of PCDD/Fs and sperm head abnormalities. Furthermore, our results revealed that individuals in the highest tertile of PCDD/Fs dietary intake exhibited higher BMI and increased consumption of fish, red meat, and its derivatives, along with decreased consumption of nuts. To the best of our knowledge, this study represents the first exploration of the associations between dietary intake of PCDD/Fs and sperm quality parameters. These results substantiate epidemiological evidence establishing a connection between ongoing exposure to PCDD/Fs and sperm quality. Despite control measures on these chemicals, PCDD/Fs may still pose a problem for health. Because of the great importance that these abnormalities could trigger, further studies assessing possible associations between EDCs and sperm morphology replicating these results are warranted. Such studies are necessary to shed light on the causes of infertility, 50 % of which are due to male factors and 12–15 % of couples worldwide are unable to conceive.

Ethics approval and consent to participate

Every participant provided informed consent through both online and written means, and the project protocol received approval from the ethical committee of Institut d'Investigacions Sanitàries Pere i Virgili (Ref. CEIM: 181/2019).

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CRediT authorship contribution statement

María Fernández de la Puente: Writing – review & editing, Validation, Methodology, Investigation. **Cristina Valle-Hita:** Writing – review & editing, Validation, Methodology, Investigation. **Nadine Khoury:** Writing – review & editing, Validation, Methodology, Investigation. **Elena Sánchez-Resino:** Writing – review & editing, Validation, Methodology, Investigation. **Carla Ramos-Rodríguez:** Writing – review & editing, Validation, Investigation. **Estefanía Davila-Cordova:** Writing – review & editing, Validation, Investigation. **Jordi Salas-Salvadó:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization. **Nancy Babio:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization. **María Martínez:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Albert Salas-Huetos:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Albert Salas Huetos reports was provided by Rovira i Virgili University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.reprotox.2025.108831.

Data availability

Data will be made available on request.

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