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Serum persistent organic pollutants and diminished ovarian reserve: a single-exposure and mixture exposure approach from a French case-control study

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1 **Serum persistent organic pollutants and diminished ovarian reserve: a single-exposure and mixture**
2 **exposure approach from a French case-control study**

3

4 **Running Title:** POP exposure and diminished ovarian reserve

5

6 M. Génard-Walton^{1,*}, C. Warembourg¹, S. Duros², F. Mercier³, T. Lefebvre⁴, A. Guivarc'h-Levêque⁵, M-T.
7 Le Martelot⁶, B. Le Bot⁷, B. Jacquemin¹, C. Chevrier¹, S. Cordier¹, N. Costet¹, L. Multigner¹, and R.
8 Garlantézec⁸

9

10 ¹Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S
11 1085, F-35000 Rennes, France

12 ²Reproductive Medicine, CHU Rennes, 35200 Rennes, France

13 ³Univ Rennes, Inserm, EHESP, Leres (Laboratoire d'étude et de recherche en environnement et santé) -
14 UMR_S 1085, F-35000 Rennes, France

15 ⁴Reproductive Medicine, CHU Nantes, 44093 Nantes, France

16 ⁵Reproductive Medicine, Clinique de La Sagesse, 35043 Rennes, France

17 ⁶Reproductive Medicine, CHU Brest, 29609 Brest, France

18 ⁷Univ Rennes, EHESP, Inserm, Irset (Institut de recherche en santé, environnement et travail) - UMR_S
19 1085, F-35000 Rennes, France

20 ⁸Univ Rennes, CHU Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail)
21 - UMR_S 1085, F-35000 Rennes, France

22

23 *Correspondence address. E-mail: maximilien.genard-walton@inserm.fr

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25

26 Abstract

27

28 Study question:

29 Are persistent organic pollutants (POPs) associated with a diminished ovarian reserve (DOR) in women of
30 reproductive age?

31 Summary answer:

32 Amongst 17 POPs detected in over 20% of serum samples, only p,p'-DDE was significantly associated with
33 an increased risk of DOR, and β -HCH was significantly associated with a decreased risk of DOR whilst
34 mixture analyses yielded non-significant associations and did not detect any interactions between POPs.

35 What is known already:

36 Animal studies have shown that several POPs can alter folliculogenesis and increase follicle depletion.
37 However, only a few studies have been conducted in humans, with small sample sizes and inconsistent
38 results.

39 Study design, size, duration:

40 Our study included 138 cases and 151 controls from the AROPE case-control study. Study participants
41 were women between 18 and 40 years of age recruited amongst couples consulting for infertility in four
42 fertility centres in western France between 2016 and 2020.

43 Participants/materials, setting, methods:

44 Cases of DOR were defined as women with AMH levels ≤ 1.1 ng/mL and/or AFC < 7 , and controls were
45 women with AMH levels between 1.1 and 5 ng/mL and AFC ≥ 7 , without genital malformations and with
46 a menstrual cycle length between 26 and 35 days. A total of 43 POPs (including 15 organochlorine
47 pesticides, 17 polychlorinated biphenyls and 9 polybromodiphenylethers) were measured in the serum at
48 inclusion into the study. We conducted logistic regression adjusted for potential confounders using a
49 directed acyclic graph (DAG) to study the effect of each POP on DOR as single exposures, and used
50 Bayesian kernel machine regression (BKMR) to measure the mixture effect of POPs on DOR.

51 Main results and the role of chance:

52 Of the 43 POPs, 17 were detected in over 20% of the serum samples. In the single-exposure multivariate
53 logistic regressions, p,p'-DDE (median 165.0 IQR 161.0 ng/L in controls) as a continuous exposure was
54 significantly associated with an increased risk of DOR (OR 1.39, 95% CI 1.10 - 1.77) and non-significantly
55 associated with an increased risk of DOR for the second and third tertiles (OR 1.46, 95% CI 0.74 - 2.87,
56 and OR 1.72, 95% CI 0.88 - 3.37, respectively). β -HCH (median 24.2 IQR 21.5 ng/L in controls) was
57 significantly associated with a decreased risk of DOR when β -HCH was treated as a continuous exposure
58 (OR 0.63, 95% CI 0.44 - 0.89) and for the third tertile of exposure (OR 0.43, 95% CI 0.21 - 0.84) and non-
59 significantly associated with a decreased risk of DOR for the second tertile (OR 0.77, 95% CI 0.42 - 1.42).
60 All sensitivity analyses confirmed our results. BKMR showed similar associations for single exposures but
61 found no significant associations for the total mixture effect. In addition, the BKMR results did not suggest
62 any interactions between POPs.

63 Limitations, reasons for caution:

64 Controls were recruited amongst infertile couples and thus may not be representative of all women of
65 reproductive age. However, their POP concentrations were in the same range as in the general French
66 population.

67 Wider implications of the findings:

68 This study is the first to examine the associations between serum POPs and DOR. The well-recognised
69 anti-androgenic properties of p,p'-DDE and estrogenic properties of β -HCH could explain these
70 associations of opposite direction. If these results are replicated elsewhere, this could have an impact on
71 fertility prevention messages and help in understanding the impact of POPs on the female reproductive
72 system.

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75 conflicts of interest to declare.

76 **Trial registration number:** N/A.

77

78 **Keywords:** Ovarian Reserve; Female Infertility; Persistent Organic Pollutants; Environmental Exposure;
79 Pesticides; Polychlorinated Biphenyls.

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85 Introduction

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87 Ovarian reserve is defined as the number and quality of primordial follicles in both ovaries (Richardson *et al.*, 2014). This stock of follicles is constituted in utero, where it can reach up to seven million, but
88 *al.*, 2014). This stock of follicles is constituted in utero, where it can reach up to seven million, but
89 subsequently decreases to one to two million at birth and thereafter to 1,000, on average, as women
90 reach menopause (Hansen *et al.*, 2008). Currently, the two most accurate indirect measurement methods
91 to evaluate the ovarian reserve in clinical practice are based on the antral follicle count (AFC) and anti-
92 Müllerian hormone (AMH) levels (Ferraretti *et al.*, 2011). The term diminished ovarian reserve (DOR) is
93 used to describe women of reproductive age whose ovarian pool, which reflects a woman's reproductive
94 potential (Tal and Seifer, 2017), is smaller than that of other women of the same age. It is one of the most
95 frequent pathologies diagnosed in women consulting for infertility (Pastore *et al.*, 2018) and is of
96 particular concern, as the mean age of conception is globally increasing (Datta *et al.*, 2016; Schmidt *et al.*,
97 2012; Vander Borgh and Wyns, 2018). DOR is associated with a poor response to ovarian stimulation (Yun
98 *et al.*, 2017) and is possibly associated with recurrent pregnancy loss (Bunnewell *et al.*, 2020). Several risk
99 factors, such as age, smoking, body-mass index (BMI), and a history of chemotherapy and radiotherapy,
100 are known to be associated with ovarian reserve indicators (de Angelis *et al.*, 2020; Moslehi *et al.*, 2018;
101 Spears *et al.*, 2019). However, the role of environmental pollutants on the ovarian reserve is less well
102 understood (Jurewicz *et al.*, 2020; Mínguez-Alarcón and Gaskins, 2017; Souter *et al.*, 2013). Amongst
103 them, persistent organic pollutants (POPs) have attracted attention because of their widespread and
104 persistent presence, both in the environment and in humans, and their ability to interfere with hormone-
105 regulated processes (Bergman *et al.*, 2013). The main categories of POPs include organochlorine
106 pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and
107 perfluoroalkyls substances (PFASs) and the vast majority of them are considered to be endocrine
108 disrupting chemicals (EDCs) (Bergman *et al.*, 2013). They are carbon-based chemical substances that
109 bioaccumulate with half-lives in humans ranging from 10-20 years for OCPs and PCBs and 5-10 years for

110 PBDEs (Bu *et al.*, 2015; Ritter *et al.*, 2011). Most of these chemicals have been banned from production
111 and trade in large parts of the world for up to several decades. Nonetheless, POPs are still detected in
112 humans worldwide. The main source of exposure to OCPs, PCBs and PBDEs, for humans is food (Bergman
113 *et al.*, 2013; Djien Liem *et al.*, 2000; Guo *et al.*, 2019) but these pollutants can also be found in the air
114 (Castro-Jiménez *et al.*, 2017; Hung *et al.*, 2005) and in indoor dust (Roosens *et al.*, 2010). Animal studies
115 have reported associations between exposure to OCPs (dichlorodiphenyltrichloroethane [DDT], γ -
116 hexachlorocyclohexane [γ -HCH] and hexachlorobenzene [HCB]) and impaired oocyte maturation
117 (Lindenau *et al.*, 1994) and folliculogenesis (Alvarez *et al.*, 2000) and between in-utero exposure to
118 selected POPs (PBDE 47 and 99, Aroclor 1016 [a commercial PCB mixture] and hexabromocyclododecane
119 [HBCD]) and a decreased number of follicles (Baldrige *et al.*, 2003; Ema *et al.*, 2008; Lilienthal *et al.*, 2006;
120 Talsness *et al.*, 2008). Moreover, in-vitro studies have shown impacts of OCPs (γ -HCH, DDT and
121 dichlorodiphenyldichloroethylene [DDE]) and PCB 126 on oocyte maturation (Alm *et al.*, 1998; Krogenæs
122 *et al.*, 1998) and folliculogenesis (Holloway *et al.*, 2007). In humans, several OCPs including p,p'-DDE, β -
123 HCH and HCB, multiple PCB congeners (e.g. 118, 138, 153, 170, 180, 183, 187) and PBDEs congeners (e.g.
124 100, 153) have been associated with a delayed time-to-pregnancy (Chevrier *et al.*, 2013; Kahn *et al.*, 2021;
125 Kim *et al.*, 2019) and a few studies have investigated the effects of POPs on indicators of the ovarian
126 reserve, such as AMH and AFC, treated as continuous variables. In a population of 50 pregnant women,
127 one study (Björvang *et al.*, 2021) found associations between higher POP levels (HCB, trans-nonachlor,
128 PCB 74 and 99) and decreased non-growing follicle densities measured in ovarian cortical tissue, which
129 disappeared for all POPs when they were adjusted for lipid levels, except for HCB. In the same study, no
130 associations were observed with serum AMH in the first trimester of pregnancy. In a subsequent paper
131 investigating 30 POPs in a cohort of 185 women undergoing assisted reproductive technologies (Björvang
132 *et al.*, 2022), the authors also found higher HCB concentrations (wet-weight) to be the only POP measured
133 in serum to be associated with lower AMH levels, whereas none were associated with AFC. Furthermore,
134 in a study of 420 null-parous women aged 20 to 30 years from a rural community in South Africa

135 (Whitworth *et al.*, 2015), p,p'-DDT and p,p'-DDE were not associated with AMH levels. Finally, in a pilot
136 study of 32 women, Bloom *et al.* (2017) identified associations between high levels of three PCBs (namely
137 PCB 151, 170 and 180), amongst 45 POPs measured in follicular fluid, and a lower number of antral
138 follicles. In summary, there are inconsistent results concerning the relationship between POPs and
139 continuous indicators of the ovarian reserve. Here, we aimed to investigate the associations between
140 serum concentrations of selected POPs and DOR defined using internationally recognised thresholds
141 among women consulting for couple infertility in North-Western France.

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143

144 **Materials and methods**

145

146 **Population**

147 We used data from the AROPE case-control study (Altération de la réserve ovarienne : étude du rôle de
148 l'exposition aux perturbateurs endocriniens persistants et aux solvants organiques), which included
149 women between the ages of 18 and 40 amongst couples consulting for infertility in four fertility centres
150 in the region of Bretagne,– Pays de Loire in north-western France (Rennes University Hospital, Nantes
151 University Hospital, Brest University Hospital, and La Sagesse, a semi-private clinic based in Rennes)
152 between November 2016 and February 2020. All women consulting in these centres underwent a
153 standardized endovaginal antral follicle count by a trained gynaecologist between days 2 and 4 of a
154 spontaneous menstrual cycle (Broekmans *et al.*, 2010) as well as an AMH measurement performed at
155 inclusion (36.2% of cases and 31.8% of controls) or within the year prior to the inclusion for women who
156 had already started consulting in the clinics (63.8% of cases and 68.2% of controls). AMH levels were
157 measured using an automated Roche Elecsys immunoassay system in all centres, excepted for Rennes
158 University Hospital, where the automated Beckman-Coulter Access Dxi assay was used (van Helden and
159 Weiskirchen, 2015). Cases of DOR were defined using the threshold values for the AFC and AMH levels
160 proposed in the European Society of Human Reproduction and Embryology (ESHRE) consensus Bologna
161 criteria (Ferraretti *et al.*, 2011). Hence, cases were women presenting a number of antral follicles lower
162 than 7 and/or a blood level of AMH lower or equal to 1.1 ng/mL. Controls were selected by frequency
163 matching per centre and per five-year age group among women whose fertility assessment was strictly
164 normal (AMH levels between 1.1 and 5 ng/mL and AFC \geq 7, without genital malformation and with a
165 menstrual cycle between 26 and 35 days). The non-inclusion criteria for cases and controls were
166 endometriosis, polycystic ovary syndrome, a history of adnexal surgery, a history of cancer with
167 chemotherapy or radiotherapy, a genetic or chromosomal syndrome (Turner or fragile X), morbid obesity
168 (BMI \geq 35 kg/m²), and women subject to legal protection or deprived of their liberty. At inclusion, a

169 gynaecologist collected medical information and the participants completed a self-administered
170 questionnaire that covered their social and demographic characteristics, personal habits and lifestyle.
171 Blood serum was also collected at inclusion to measure the chemicals of interest. In total, 149 cases and
172 161 controls were included in the AROPE study, with a participation rate of 65% for cases and 75% for
173 controls amongst women who were invited to participate.

175 **Assessment of exposure**

176 After clotting, blood samples were centrifuged and serum samples were separated by centrifugation,
177 transferred immediately to propylene vials with a Teflon stopper, and frozen at -20°C. Samples were
178 identified solely by a unique sample code and transferred, on dry ice, to the Leres (Laboratoire d'Etude et
179 de Recherche en Environnement et Santé) laboratory in Rennes, France. All laboratory personnel were
180 blind to the case/control status. POP measurements were carried out on 138 cases and 151 controls, as
181 there was an insufficient quantity of serum for 11 cases and 10 controls. A participant flowchart is
182 available in Supplementary Figure S1 and the study design is outlined in Supplementary Figure S2. A gas
183 chromatograph (GC 7890B, Agilent Technologies, Santa Clara, California, United States) coupled with a
184 triple quadrupole mass spectrometer (7010B Triple Quadrupole GC/MS, Agilent Technologies) was used
185 to determine the serum concentrations of 43 POPs: 15 organochlorines (aldrin, dieldrin, o,p'-DDE, o,p'-
186 DDT, p,p'-DDE, p,p'-DDT, α -endosulfan, β -endosulfan, heptachlor, cis-heptachlor epoxide, trans-
187 heptachlor epoxide, α -HCH, β -HCH, γ -HCH, HCB, 17 PCB congeners (28, 31, 52, 74, 99, 101, 118, 138, 153,
188 163, 170, 180, 183, 187, 194, 196, and 203), 9 PBDEs congeners (28, 47, 66, 85, 99, 100, 153, 154, and
189 183), one polybromobiphenyl congener (153) and HBCD (as a mixture of α , β and γ isomers). A detailed
190 description of the method, including quality assurance and quality control, is provided in Supplementary
191 File S1 and Supplementary Tables SI and SII. Concentrations are reported as wet weight (units of
192 micrograms per litre) and the limits of detection (LOD) ranged from 0.0025 $\mu\text{g/L}$ for most POPs to 2.5 $\mu\text{g/L}$
193 for PBDE 183. Total cholesterol (TC) and triglyceride (TG) levels were enzymatically measured in serum

194 samples (in mmol/L). After conversion to g/L, total lipid levels were calculated as $2.27*TC + TG + 0.623$
195 (Phillips *et al.*, 1989).

196

197 **Statistical analyses**

198 All POP concentrations in our study were standardised using total serum lipids, as recommended by
199 O'Brien *et al.* (2016) (O'Brien *et al.*, 2016). POP concentrations were then log-2 transformed when treated
200 as a continuous variable. POPs detected in fewer than 20% of samples were not included in the analyses.
201 For POPs detected in between 20% and 50% of samples, exposure was dichotomised into two groups
202 (detected versus not detected). For others (POPs detected in over 50% of samples), concentrations below
203 the limit of detection (LOD) were replaced by combining the results of five imputations using maximum
204 likelihood estimations (Cole *et al.*, 2009) and were categorised in terciles. For these POPs, analyses were
205 also performed on continuous exposure. Associations between cases and control status and exposures
206 variables were studied using unconditional logistic regression as recommended for frequency matching
207 (Kuo *et al.*, 2018; Pearce, 2016). The logistic regression results are presented as odds ratios (OR) with their
208 corresponding 95% confidence intervals (CI). We reviewed the relevant literature to develop a directed
209 acyclic graph (DAG) (Shrier and Platt, 2008) (Supplementary Figure S3), which was used to determine the
210 minimally sufficient adjustment sets of variables for regression to estimate the total effects of the studied
211 exposure on the outcome. Based on this DAG, our models were systematically adjusted for age
212 (continuous), education level (high school or lower, high school + 2 years of university, high school + 3
213 years of university and above), BMI (continuous) and season of inclusion (autumn, winter, spring,
214 summer). This set of adjustments was supplemented by an a priori adjustment on centre of inclusion.
215 Covariates with missing data were imputed using multiple imputation by chained equations (five sets)
216 (Kristman *et al.*, 2005). Additionally, we conducted sensitivity analyses using different definitions for
217 cases: one based on the AFC outcome only (AFC < 7), one based on the AMH outcome only (AMH levels ≤
218 1.1 ng/mL), and one based on the combination of both the AFC and AMH outcomes (AFC < 7 and AMH

219 levels ≤ 1.1 ng/mL). In addition, as suggested by Ferraretti & Gianaroli (2014), we performed a sensitivity
220 analysis in which cases were defined as AFC < 5 or AMH levels ≤ 0.7 ng/mL (Ferraretti and Gianaroli, 2014).
221 All sensitivity analyses were performed using the same original group of controls (N=151). Finally, we
222 carried out two sensitivity analyses, one restricted to women who had not undergone any prior ovarian
223 stimulation to remove the potential impact of previous ovarian stimulation on our models, and another
224 restricted to women whose outcomes were measured at inclusion. Furthermore, we used Bayesian kernel
225 machine regression (BKMR) (Bobb *et al.*, 2015) for POPs with a detection rate above 50% to evaluate the
226 mixture effect of POPs on DOR. BKMR is a non-parametric Bayesian method that can flexibly model the
227 association between multiple exposures and an outcome using a kernel function. This method allows for
228 the integration of non-linear associations, as well as interactions between exposures. POPs were
229 organised into groups of POP families. Hierarchical variable selection was performed to estimate the
230 posterior inclusion probabilities of the groups and individual POPs. The model was fit with 10,000
231 iterations using a Markov chain Monte Carlo (MCMC) algorithm. One individual was considered to exert
232 an overly large leverage on the mixture effect and was therefore removed from the BKMR analysis. All
233 analyses were performed using R (version 4.0.4). The threshold for significance for all analyses was 5%.

234

235 **Ethics approval**

236 The study was approved by the French ethical board for human participation in research study on health
237 (authorisation n°2016-A00307-44) and the French national security agency for medicine and health
238 products (ANSM). All participants provided informed consent for their participation in the study.

239

240

241 **Results**

242

243 **Population description**

244 The participant characteristics can be found in Table I. The mean age was 33.8 (\pm 4.0) years for cases and
245 32.4 (\pm 4.1) years for controls. Most study participants (66.6% for cases and 64.3% for controls) had an
246 education level equivalent to high school + 2 years university or above. Their ethnic origin was mostly
247 European (87.4% of cases and 90.8% of controls). Half of the women were never smokers (52.1% in cases
248 and 51.1% in controls) and close to two thirds of the participants were women with primary infertility
249 (60.9% in cases and 58.3% in controls).

250

251 **Serum concentrations of POPs**

252 Table II shows the detection rates and the serum concentrations of POPs in the control and case groups.
253 Out of the 43 persistent organic pollutants, 17 were detected in over 20% of samples and 15 were
254 detected in over 90% of samples. The POP with the highest median serum concentration was p,p'-DDE
255 (28.9 ng/g lipid base), followed by PCB 153 (21.1 ng/g lipid base), PCB 180 (15.2 ng/g lipid base) and HCB
256 (7.4 ng/g lipid base). Correlation coefficients (Supplementary Figure S4) between PCBs were between 0.4
257 and 1, except those for PCB 28, which were lower. OCPs were correlated together (between $\rho = 0.3$ and
258 $\rho = 0.6$), except for p,p'-DDT, which only correlated with p,p'-DDE ($\rho = 0.6$). p,p'-DDE, HCB and β -HCH
259 correlated moderately with PCBs ($0.3 < \rho < 0.6$), with the exception of PCB 28.

260

261 **Associations between POP exposures and DOR**

262 Associations between tercile and continuous POP exposures and DOR are presented in Table III. In
263 multivariate logistic regressions, p,p'-DDE as a continuous exposure was significantly associated with an
264 increased risk of DOR (OR 1.39, 95% CI 1.10 - 1.77) and was non-significantly associated with an increased
265 risk of DOR for the second and third terciles (OR 1.46, 95% CI 0.74 - 2.87 and OR 1.72, 95% CI 0.88 - 3.37,

266 respectively). By contrast, β -HCH was significantly associated with a decreased risk of DOR when β -HCH
267 was treated as a continuous exposure (OR 0.63, 95% CI 0.44 - 0.89) and for the third tercile of exposure
268 (OR 0.43, 95% CI 0.21 - 0.84) and was non-significantly associated with a decreased risk of DOR for the
269 second tercile (OR 0.77, 95% CI 0.42 - 1.42). There were no significant associations for any other POPs.

271 **Sensitivity analyses**

272 The associations found in the main analysis for p,p'-DDE and β -HCH were confirmed in all sensitivity
273 analyses (Supplementary Tables SIII, SIV, SV, SVI, SVII and SVIII). In addition, PCB 118 was significantly
274 associated with an increased risk of DOR in multivariate regressions when cases were defined as AFC < 7
275 for the second tercile of exposure (OR 2.98, 95% CI 1.09 - 8.11).

277 **Associations between the POP mixture and DOR: BKMR analysis**

278 POPs were organised into two groups: the OCP group and the PCB group. The combined effect of POPs on
279 DOR in BKMR analysis is shown in Figure 1. The group posterior inclusion probabilities (PIP) (Table IV)
280 showed that the model favours the inclusion of the OCP group more (96.9%) than the PCB group (31.8%).
281 The OCP PIP was mainly driven by β -HCH (91.4%) and then p,p'-DDE (6.8%). The strongest PIPs in the PCB
282 group were PCB 99 (17.6%) and PCB 183 (11.5%). As we can observe in Figure 2, all plots, except those for
283 p,p'-DDE and β -HCH, showed no impact of the exposure on the probability of having DOR. The plot for
284 p,p'-DDE showed a quasi-linear relationship with DOR, whereas β -HCH showed a non-linear relationship.
285 Nonetheless, the relationship with β -HCH indicates stabilisation of the effect on DOR in the higher
286 quantiles of exposure. Furthermore, the overall mixture effect was Beta = -0.14, 95% CI -0.36 - 0.06, when
287 all exposures were fixed at the 75th percentile compared to when they were all fixed at the 50th
288 percentile. We did not detect any interaction between different POPs when investigating the individual
289 effect of each POP and comparing the 75th to the 25th percentile of exposure when all other POPs were
290 fixed at the 25th, 50th, and 75th percentiles (Figure 3).

291 **Discussion**

292

293 Amongst 17 POPs detected in more than 20% of our serum samples, only two, p,p'-DDE and β -HCH, were
294 associated with DOR. p,p'-DDE was significantly associated with an increased risk of DOR, whereas β -HCH
295 was significantly associated with a decreased risk of DOR, both in a dose-dependent manner. These results
296 were stable in all sensitivity analyses including when DOR was only defined using AFC or AMH only. The
297 BKMR analysis showed no significant mixture effect and an absence of interactions between POPs.

298 To the best of our knowledge, our study is the first to analyse the relationship between POPs and DOR
299 (defined in this study as AFC < 7 and/or AMH \leq 1.1 ng/L using ESHRE thresholds). However, four previous
300 studies (Björvang *et al.*, 2021, 2022; Bloom *et al.*, 2017; Whitworth *et al.*, 2015) investigated the
301 relationship between p,p'-DDE and AMH levels and/or AFC as continuous variables and did not report any
302 significant associations. Nevertheless, the populations in two of these studies were quite different
303 compared to ours, namely, young rural South African women (Whitworth *et al.*, 2015) and pregnant
304 women (Björvang *et al.*, 2021). Two studies also had limited sample sizes (50 women for Björvang *et al.*
305 (2021) and 32 women for Bloom *et al.* (2017)). Furthermore, studies that previously attempted to look at
306 the role of β -HCH on AMH levels or AFC were unable to detect the molecule in enough samples to carry
307 out the analyses (Björvang *et al.*, 2021, 2022; Cole *et al.*, 2006).

308 Our results regarding p,p'-DDE are in accordance with previous in-vitro and in-vivo experimental data. In
309 primary cultures of human granulosa cells, p,p'-DDE was reported to increase FSH stimulation of
310 aromatase activity, resulting in the overproduction of estradiol, leading to an increase in follicular
311 recruitment and the acceleration of follicular depletion (Younglai *et al.*, 2004). In addition, p,p'-DDE was
312 shown to reduce the synthesis of vascular endothelial growth factor (VEGF) and insulin-like growth factor-
313 1 (IGF-1) of granulosa cells in primary human cultures and rat ovaries (Holloway *et al.*, 2007), which may
314 in turn alter ovarian follicular development. Moreover, Chedrese and Feyles (2001), reported that p,p'-
315 DDE, but not estradiol, can decrease progesterone synthesis in hamster ovary cells. These results highlight

316 that the effect of p,p'-DDE on ovarian cells can be mediated by non-estrogenic activities, which may
317 include its well-known anti-androgenic properties (You *et al.*, 1998, 1999). On the other hand, β -HCH has
318 been shown to exert estrogen-like effects in MCF-7 human breast cells (Hatakeyama *et al.*, 2002) and
319 uterotrophic responses in ovariectomized mice (Bigsby *et al.*, 1997). Furthermore, adult rats exposed to
320 high exposure levels of β -HCH (≥ 50 mg/kg) showed atrophy of the ovaries, impaired oogenesis and focal
321 hyperplasia and metaplastic changes of the endometrial epithelium, which could be due to an estrogenic
322 action of β -HCH (Van Velsen *et al.*, 1986). We believe that the opposite direction of the associations
323 between p,p'-DDE and β -HCH and DOR observed in our study may be explained, at least partially, by the
324 different mechanisms and hormonal actions of these chemicals, i.e., the anti-androgenic properties of
325 p,p'-DDE and the oestrogenic properties of β -HCH. The opposite direction of the association between
326 POPs with anti-androgenic and estrogenic properties has been previously reported in other epidemiology
327 studies, notably regarding time to pregnancy (Cohn *et al.*, 2003) and prostate cancer (Emeville *et al.*,
328 2015). Other studies are needed to replicate these observations on DOR as well as mechanistic studies to
329 better understand these intricate phenomena.

330 This study has several limitations. POPs were either measured at the time of inclusion or less than one
331 year after the ovarian reserve evaluations. There is therefore a possibility for reverse causation of the
332 associations. However, POPs, especially OCPs and PCBs, are persistent chemicals with long half-lives that
333 accumulate in fat tissues over time (Bu *et al.*, 2015) and their concentrations in human serum is hence
334 considered as a marker of long-term exposure. Additionally, human exposure to POPs has gradually
335 decreased over time (Bányiová *et al.*, 2017; Schoeters *et al.*, 2017). Another study (Stubbleski *et al.*, 2018)
336 also found that after a 5-year follow-up, POP concentrations in an elderly population decreased
337 significantly with most reductions being in the 30–40% range but with lower declines for women
338 compared to men. Nevertheless correlations between POPs between time points remain high (Bányiová
339 *et al.*, 2017; Nøst *et al.*, 2019), indicating that the overall effect (change in risk of DOR for one unit increase
340 in POP concentrations) should be stable if POP concentrations were lower at the time of sampling.

341 In addition, if the infertility status of the control partners was related to POP levels as shown in studies on
342 POPs and semen parameters (Vested *et al.*, 2014), and exposure to POPs was shared between the
343 members of the couple, it could imply that the controls included in the AROPE study were more exposed
344 to POPs than the general population. If this was indeed the case, this phenomenon could have led to
345 reduced associations between POPs and DOR in our study. Nevertheless, Góralczyk *et al.* (2015) reported
346 low correlations of POP concentrations between partners living together. Moreover, the POP
347 concentration levels of the AROPE controls are comparable to the levels of the general population. PCB
348 levels were slightly lower than those reported in a French mother-child cohort (ELFE, 2011) (Dereumeaux
349 *et al.*, 2016) and OCP concentrations were comparable to concentrations found in a French national cross-
350 sectional study (ESTEBAN, 2014-16) (Santé Publique France, 2021).

351 We chose, a priori, to study the relation between POPs and DOR rather than indicators of ovarian reserve
352 treated as continuous variables such as in previous published studies in order to use a more clinically
353 pertinent outcome. Additionally, doing so allowed us to ensure we could recruit more women with lower
354 ovarian reserves, but with the cost of losing the added statistical power of modeling on continuous
355 variables. Our definition of DOR uses threshold levels for AFC and AMH levels that were proposed by the
356 Bologna ESHRE consensus for the purpose of predicting a poor ovarian response (POR). However, many
357 fertility centres also use this definition to diagnose DOR (Pastore *et al.*, 2018). We performed several
358 sensitivity analyses using various definitions of the outcome to limit the risk of DOR misclassification: AFC
359 < 7 , AMH levels ≤ 1.1 ng/mL, and AFC < 7 & AMH levels ≤ 1.1 ng/mL. Furthermore, Ferraretti & Gianaroli
360 (2014) suggested modifying the Bologna criteria to adjust the cut-off values. We therefore implemented
361 an additional sensitivity analysis in which DOR was defined as AFC < 5 and/or AMH levels ≤ 0.7 ng/mL. The
362 results of all sensitivity analyses were consistent with our main findings. Nonetheless, there is still a
363 possibility that we missed a critical threshold which could be responsible for a lack of significant results in
364 our findings. Finally, the analytical system for the determination of AMH levels was different in one centre
365 (Rennes University Hospital), which used the Beckman-Coulter Access assay, whereas the other centres

366 used AMH Roche Elecsys Assays. Nevertheless, these assay systems have been shown to provide highly
367 correlated results in comparative studies ($r = 0.903$ (Li *et al.*, 2016) and $r = 0.991$ (van Helden and
368 Weiskirchen, 2015)). Any residual difference should have been accounted for through our adjustment on
369 centres of inclusion. In addition, the AMH concentrations used in this study were measured within the
370 year before inclusion. This could result in a non-differential measurement bias, as AMH levels decrease
371 over time. Despite this imprecision, the results of our sensitivity analysis restricted to women with no
372 prior ovarian stimulation (Supplementary Table SVII) or women whose outcomes were measured at
373 inclusion (Supplementary Table SVIII) did not deviate from the main results of our study.

374 Given the number of pollutants analysed in this study, it is possible that our findings are impacted by the
375 occurrence of false positives or negatives. However, the objective of this study was to provide signals to
376 be confirmed in further studies rather than to reach definite conclusions. All analyses were also planned
377 a priori and consequently, we decided not to correct our results for false discoveries (Bender and Lange,
378 2001; Goeman and Solari, 2011). Finally, our results were consistent in all our sensitivity analyses.

379 Our study also has several strengths. The AROPE study recruited cases and controls from four of the most
380 prevalent fertility centres in north-western France. The French national health insurance funds infertility
381 consultations and the first four in-vitro fertilisation attempts until the age of 43 (National Health
382 Insurance, 2021), which may limit a potential selection bias when recruiting patients in fertility centres.
383 However, it is still possible that the study participants could have higher levels of education than that of
384 the general population, as shown in a British study on the prevalence of infertility and help seeking, in
385 which the authors reported that women with lower levels of education are less likely to seek help (Datta
386 *et al.*, 2016). In addition, although the AROPE study's participation rate was 65% for cases and 75% for
387 controls, there were no differences between the patients included or not in our study in terms of age,
388 BMI or parity (data not shown). Another strength of our study, as mentioned above, is that we performed
389 multiple sensitivity analyses varying our case definition using pertinent thresholds to avoid issues of
390 measurement bias.

391 Furthermore, we used an a priori adjustment strategy using DAGs to limit over-adjustments (Shrier and
392 Platt, 2008). Moreover, all our analyses were based on lipid-normalised POP levels (standardisation). We
393 chose this strategy rather than that of lipid-adjusted models in light of results from a simulation study by
394 O'Brien et al (2016), which investigated various scenarios for the relationship between POP exposure, lipid
395 levels, and outcomes and standardisation and showed that standardisation performed best under the
396 causal scenario of our study, i.e., when considering whether recent fat intake affects serum exposure
397 concentrations and when other covariates affect both exposure concentrations and the outcome.

398 Lastly, we were able to analyse the effects of individual POPs on DOR as well as the effect of POPs as a
399 mixture using BKMR. This method allowed us to include non-linear associations and check for interactions
400 between pollutants. Evaluating the mixture effect is of particular importance because chemicals could
401 either show no significant associations when studied individually and yet have an effect when treated as
402 a mixture. Alternatively, they could have individual effects and either have an even larger impact as a
403 mixture or mitigate each other's impacts (Le Magueresse-Battistoni *et al.*, 2018).

404 Although we found some evidence of associations with POP levels in adulthood, other windows of
405 exposure, especially prenatal exposure, may be critical to understanding the impact of POPs on the
406 ovarian reserve. Because the development of the ovarian reserve occurs in utero and is not resupplied
407 afterwards (Richardson *et al.*, 2014), the effects of exposure to POPs in utero on the pool of primordial
408 follicles could have long-lasting impacts. Incidentally, animal data have reported evidence for an
409 association between in-utero exposure to several POPs and a decreased number of follicles (Baldrige *et al.*
410 *et al.*, 2003; Ema *et al.*, 2008; Lilienthal *et al.*, 2006; Talsness *et al.*, 2008). Data is scarce for humans, but one
411 study (Kristensen *et al.*, 2016) found an association between prenatal p,p'-DDE levels and a reduced
412 number of follicles at 19 years of age.

413 In conclusion, we found limited but suggestive evidence of associations between serum POPs and DOR in
414 infertile couples, especially for p,p'-DDE and β -HCH, which showed opposite associations. These results
415 may be explained by possible differences in the endocrine properties of these compounds and need to be

416 replicated in further studies. In addition, future research should also focus on exploring different windows
417 of susceptibility, including preconceptional, prenatal and peripubertal exposure.

418

419

420 **Data availability:** Due to French ethical legislation regarding the privacy of individuals who participated
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428 **Authors' roles:** RG designed the initial study. SD, TL, AGL and MTLM were in charge of patient inclusion
429 in the centres. BLB and FM were responsible for the POP analyses in serum. MGW, BJ, CC, NC, SC, LM,
430 CW and RG planned the analysis. MGW and RG analysed the data and wrote the initial manuscript. All
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434 **Conflict of interest:** None to declare.

435

436

Figure legends

Figure 1. Difference in risks (and their 95% credible intervals) of diminished ovarian reserve due to exposure to the persistent organic pollutant mixture when all pollutant concentrations are fixed at different percentiles compared to when they are fixed at their median levels. The model was adjusted for age, centre, education level, body-mass index and season of inclusion.

Figure 2. Difference in risks (blue lines) and their 95% credible intervals (shaded areas) of diminished ovarian reserve at a given exposure level of each persistent organic pollutant compared to the median exposure level of that pollutant while holding all other chemical exposure at their median levels. The model was adjusted for age, centre, education level, body-mass index and season of inclusion. p,p'-DDE, p,p'-dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; β -HCH, β -hexachlorocyclohexane; PCB, polychlorinated biphenyl.

Figure 3. Effects of individual persistent organic pollutants (and their 95% credible intervals) on the risk of diminished ovarian reserve with all other pollutant exposures fixed at different quantiles (0.25, 0.50, 0.75). The model was adjusted for age, centre, education level, body-mass index and season of inclusion. p,p'-DDE, p,p'-dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; β -HCH, β -hexachlorocyclohexane; PCB, polychlorinated biphenyl.

Supplementary Figure S1. AROPE study inclusion flowchart.

Supplementary Figure S2. AROPE study inclusion process.

Supplementary Figure S3. Directed acyclic graph between persistent organic pollutants and diminished ovarian reserve.

Supplementary Figure S4. Spearman correlation plot for persistent organic pollutant with a detection frequency above 20% in the control population (N = 151).

p,p'-DDE, p,p'-dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; β -HCH, β -hexachlorocyclohexane; PCB, polychlorinated biphenyl.

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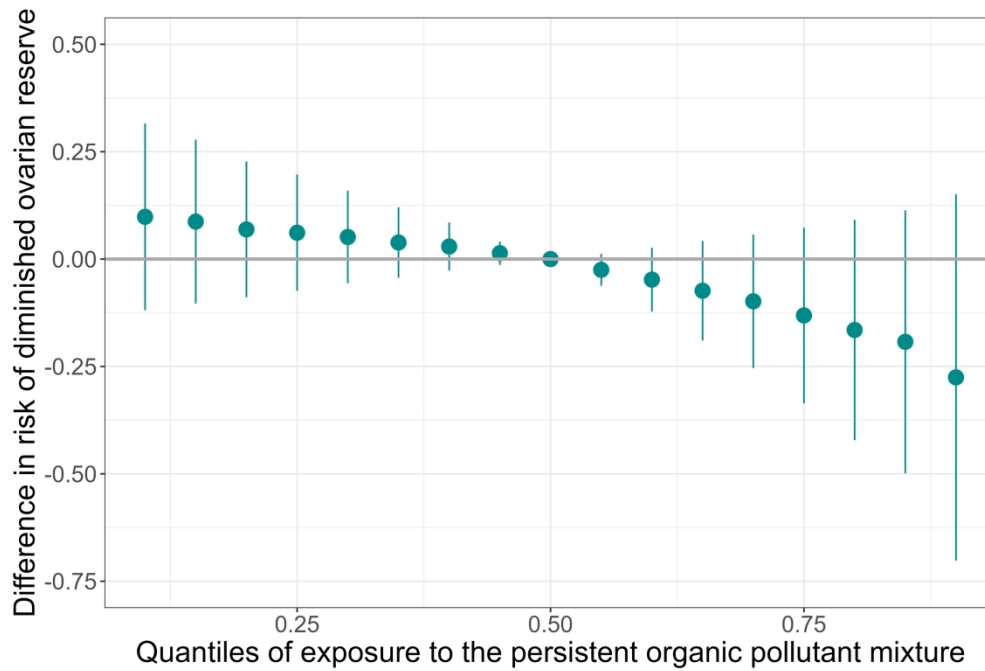


Figure 1. Mixture effect of persistent organic pollutants on diminished ovarian reserve at different percentiles of exposure compared to the 50th percentile. Difference in risk and its 95% credible interval of diminished ovarian reserve due to exposure to persistent organic pollutants when all pollutants are at different percentiles compared to when they are at their median levels. The model was adjusted for age, centre, education level, body-mass index, and season of inclusion.

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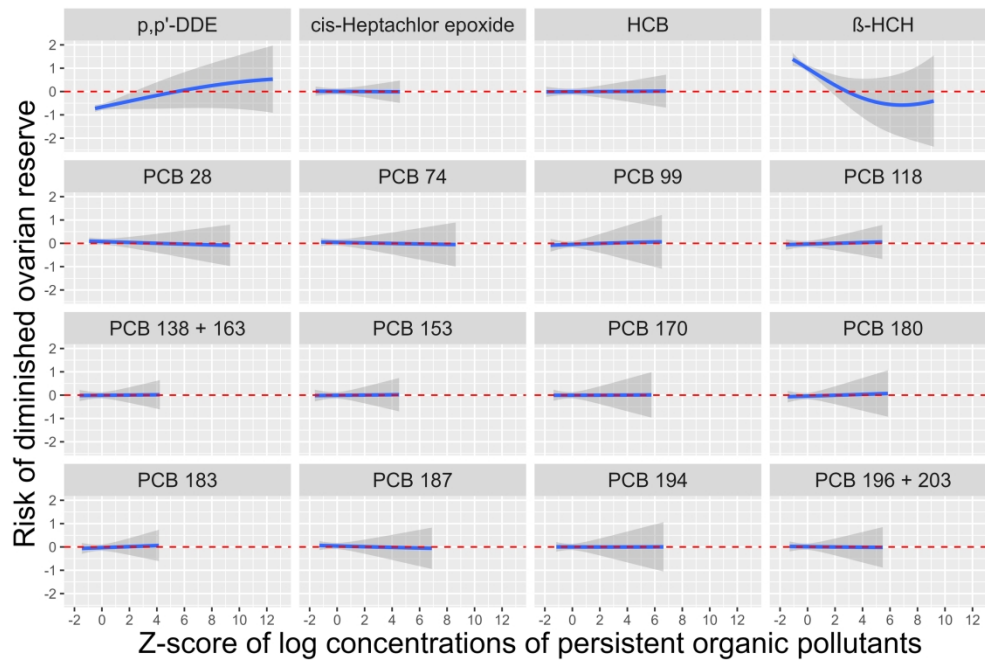


Figure 2. Mixture analysis of the exposure-response relationships between individual persistent organic pollutants and diminished ovarian reserve. Difference in risk (blue lines) and its 95% credible interval (shaded areas) of diminished ovarian reserve at a given exposure level of each persistent organic pollutant compared to the median exposure level of that pollutant while holding all other chemical exposure at their median levels. The model was adjusted for age, centre, education level, body-mass index, and season of inclusion.

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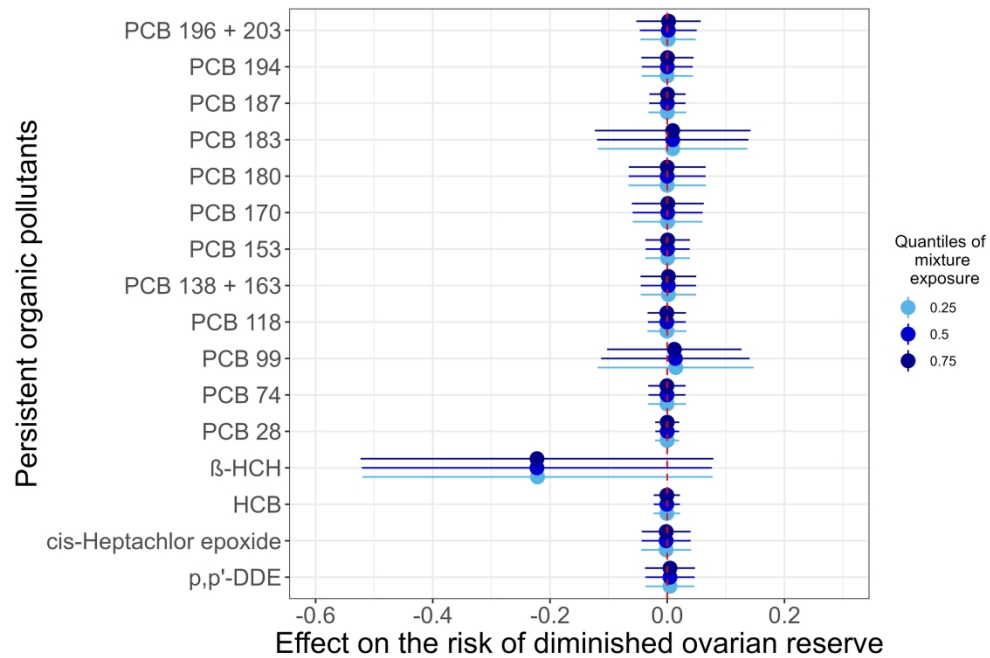


Figure 3. Effects of persistent organic pollutants on the risk of diminished ovarian reserve when the mixture exposure is fixed at different quantiles. Effect of individual persistent organic pollutants (and 95% credible intervals) on the risk of diminished ovarian reserve with all other pollutant exposures fixed at different quantiles (0.25, 0.5, 0.75). The model was adjusted for age, centre, education level, body-mass index, and season of inclusion.

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Table I. Study population characteristics (N = 138 cases and 151 controls)

Characteristic - N (%)	Cases (N = 138)	Controls (N = 151)
Centre		
Rennes University Hospital	34 (24.7)	42 (27.8)
Brest University Hospital	8 (5.8)	4 (2.6)
Nantes University Hospital	58 (42.0)	78 (51.7)
La Sagesse Clinic	38 (27.5)	27 (17.9)
Season of inclusion		
Autumn	26 (18.8)	43 (28.5)
Spring	49 (35.5)	41 (27.2)
Summer	29 (21.0)	23 (15.2)
Winter	34 (24.7)	44 (29.1)
Age		
Mean \pm SD	33.8 \pm 4.0	32.4 \pm 4.1
[18,25]	6 (4.3)	10 (6.6)
[25,30]	14 (10.2)	29 (19.3)
[30,35]	52 (37.7)	65 (43.0)
[35,40]	66 (47.8)	47 (31.1)
Ethnic background		
Europe	111 (87.4)	128 (90.8)
Outside Europe	16 (12.6)	13 (9.2)
Missing data	11	10
Education		
High school or lower	42 (33.4)	49 (35.8)
High school +2 years of university	25 (19.8)	22 (16.1)
High school +3 years or higher of university	59 (46.8)	66 (48.2)
Missing data	12	14
Body-Mass Index (kg/m²)		
Mean \pm SD	22.9 \pm 3.4	23.6 \pm 3.8
< 20	24 (17.4)	27 (18.0)
[20,25]	83 (60.2)	75 (50.0)
[25,30]	25 (18.1)	36 (24.0)
[30,35]	6 (4.3)	12 (8.0)
Missing data	0	1
Infertility status		
Primary	84 (60.9)	88 (58.3)
Secondary	54 (39.1)	63 (41.7)
Parity		
No children	84 (60.9)	88 (58.3)
1 child	33 (23.9)	33 (21.9)
\geq 2 children	21 (15.2)	30 (19.9)
Anti-müllerian hormone levels (ng/mL)		
Mean \pm SD	0.61 \pm 0.34	2.94 \pm 1.00
Antral follicle count		
Mean \pm SD	8 \pm 5	21 \pm 9
Follicle-stimulating hormone (IU/L)		
Mean \pm SD	12.3 \pm 12.4	6.7 \pm 2.1
Missing data	4	3
Luteinising hormone (IU/L)		

2

Mean ± SD	7.7 ± 8.3	5.8 ± 2.4
Missing data	9	4
Estradiol (pg/mL)		
Mean ± SD	44.3 ± 20.8	37.0 ± 16.1
Missing data	19	8
Age at menarche		
Mean ± SD	12.9 ± 1.6	13.0 ± 1.7
Missing data	17	19
Smoking status		
Never smoker	61 (48.4)	68 (48.6)
Past smoker	46 (36.5)	49 (35.0)
Current smoker	19 (15.1)	23 (16.4)
Missing data	12	11
Cigarette pack-years		
Non smoker	61 (52.1)	67 (51.1)
[0,3]	26 (22.2)	18 (13.7)
[3,6]	14 (12.0)	16 (12.2)
≥ 6	16 (13.7)	30 (22.9)
Missing data	21	20
Alcohol (number of drinks per week)		
Non drinker	36 (29.5)	22 (16.2)
[0,7]	78 (63.9)	101 (74.3)
> 7	8 (6.6)	13 (9.6)
Missing data	16	15

Abbreviations: N, number of subjects; SD, standard deviation.

Table II. Serum persistent organic pollutant (POP) concentrations in ng/L (ng/g lipid base) in the AROPE study (N = 138 cases and 151 controls)

POPs ¹	Groupe	LOD (ng/L)	Detection N (%)	Minimum	Percentiles			Maximum
					25	50	75	
Organochlorine pesticides								
Dieldrine	Controls	25	26 (17.2)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	121 (21.3)
	Cases	25	18 (13)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	82 (14.2)
o,p'-DDE	Controls	2.5	1 (0.7)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	2.7 (0.5)
	Cases	2.5	0	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)
p,p'-DDE	Controls	10	151 (100)	25 (4.4)	111.5 (19.9)	165.0 (28.9)	272.5 (48.8)	1839 (324.2)
	Cases	10	138 (100)	41 (7)	132.5 (24.0)	202.0 (35.5)	355.2 (62.1)	7532 (1229.1)
p,p'-DDT	Controls	5	51 (33.8)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	6.2 (1.1)	186 (33.4)
	Cases	5	52 (37.7)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	7.4 (1.3)	170.3 (29)
Heptachlore	Controls	2.5	1 (0.7)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	3.6 (0.6)
	Cases	2.5	0	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)
cis-Heptachlor epoxide	Controls	5	143 (94.7)	<LOD (<LOD)	8.2 (1.5)	11.1 (2)	15.1 (2.6)	34.4 (5.3)
	Cases	5	133 (96.4)	<LOD (<LOD)	8.1 (1.6)	10.3 (1.9)	14 (2.4)	26.6 (5.5)
HCB	Controls	2.5	151 (100)	11.3 (2)	34 (6.1)	41.5 (7.4)	50.2 (9)	149 (25.7)
	Cases	2.5	138 (100)	15.6 (2.6)	35.7 (6.4)	41.7 (7.5)	47.7 (9)	108.3 (15.1)
α-HCH	Controls	2.5	6 (4)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	7.9 (1.4)
	Cases	2.5	7 (5.1)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	23.4 (3.7)

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β-HCH	Controls	2.5	151 (100)	6.4 (1.3)	16.7 (3.3)	24.2 (4.5)	38.2 (6.3)	262.6 (47.2)
	Cases	2.5	138 (100)	2.8 (0.6)	17.1 (3.1)	22.5 (4.1)	34.5 (5.9)	587.3 (110.7)
γ-HCH	Controls	2.5	12 (7.9)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	13 (2.4)
	Cases	2.5	13 (9.4)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	48.5 (7.7)
Polychlorinated biphenyls								
PCB 28	Controls	2.5	75 (49.7)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	4.2 (0.8)	32.8 (6.6)
	Cases	2.5	68 (49.3)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	4.1 (0.7)	22.1 (3.8)
PCB 52	Controls	2.5	9 (6)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	20.4 (2.5)
	Cases	2.5	8 (5.8)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	11 (1.9)
PCB 74	Controls	2.5	147 (97.4)	<LOD (<LOD)	8.2 (1.6)	11.8 (2.1)	17.2 (2.9)	106.4 (21.2)
	Cases	2.5	137 (99.3)	<LOD (<LOD)	8.6 (1.6)	12.1 (2.2)	17.3 (3)	88.5 (15.1)
PCB 99	Controls	2.5	150 (99.3)	<LOD (<LOD)	8.9 (1.6)	12.2 (2.2)	16.8 (3.1)	65.6 (13.1)
	Cases	2.5	137 (99.3)	<LOD (<LOD)	10.1 (1.8)	13.3 (2.5)	18.1 (3.2)	43.5 (7.4)
PCB 101	Controls	2.5	19 (12.6)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	9.8 (1.9)
	Cases	2.5	20 (14.5)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	16.2 (2.7)
PCB 118	Controls	2.5	151 (100)	5.3 (0.9)	17.8 (3.1)	24.3 (4.3)	35.5 (6.2)	107.9 (21.5)
	Cases	2.5	138 (100)	5.2 (1)	19.9 (3.5)	26.8 (4.9)	34.3 (6.3)	90.2 (17.3)
PCB 138+163 ²	Controls	2.5	151 (100)	14.5 (2.6)	56.6 (10.4)	76 (13.5)	105.2 (19.2)	276.1 (55)
	Cases	2.5	138 (100)	16.6 (2.8)	64.5 (11.1)	87.1 (16.7)	114.6 (19.4)	285.7 (47.8)
PCB 153	Controls	2.5	151 (100)	21.3 (3.8)	80 (15.3)	120.6 (21.1)	162.9 (29.1)	401.5 (80)
	Cases	2.5	138 (100)	24.4 (4.1)	97.9 (16.3)	130.1 (24.3)	170.4 (30.5)	452.4 (76.2)
PCB 170	Controls	2.5	151 (100)	4.5 (0.8)	17.6 (3.3)	27.2 (5.2)	43 (7.6)	137 (24.6)
	Cases	2.5	138 (100)	3.7 (0.6)	20.8 (3.9)	32.8 (6.1)	46.1 (7.8)	164.8 (30.4)

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PCB 180	Controls	2.5	151 (100)	12.3 (2.2)	50 (9.3)	78.8 (15.2)	121.9 (21.6)	362.9 (65.2)
	Cases	2.5	138 (100)	10 (1.7)	63.7 (11.6)	98.7 (18.1)	127.5 (23)	456.6 (84.2)
PCB 183	Controls	2.5	144 (95.4)	<LOD (<LOD)	4.7 (0.8)	6.6 (1.2)	9.4 (1.7)	28 (5.6)
	Cases	2.5	133 (96.4)	<LOD (<LOD)	5.5 (1)	7.9 (1.4)	10.6 (1.9)	27.1 (5.6)
PCB 187	Controls	2.5	149 (98.7)	<LOD (<LOD)	9.7 (1.7)	15.3 (2.7)	25.2 (4.5)	117.9 (21.2)
	Cases	2.5	138 (100)	3.1 (0.5)	11.5 (2.1)	18.3 (3.3)	25 (4.5)	86.1 (12.7)
PCB 194	Controls	2.5	141 (93.4)	<LOD (<LOD)	5.4 (1.1)	8.8 (1.6)	15.1 (2.6)	52.8 (9.5)
	Cases	2.5	133 (96.4)	<LOD (<LOD)	7.1 (1.4)	11.1 (2.1)	16.7 (2.9)	68.7 (12.7)
PCB 196+203 ²	Controls	2.5	141 (93.4)	<LOD (<LOD)	5.1 (0.9)	8 (1.4)	12.4 (2.1)	35.7 (7.1)
	Cases	2.5	133 (96.4)	<LOD (<LOD)	6.6 (1.2)	9.6 (1.8)	13.1 (2.3)	45.8 (8.4)
Polybromodiphenylethers								
BDE 28	Controls	2.5	8 (5.3)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	54.4 (8)
	Cases	2.5	3 (2.2)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	3.6 (0.6)
BDE 47	Controls	5	4 (2.6)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	131.1 (19.2)
	Cases	5	7 (5.1)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	176.2 (29.9)
BDE 66	Controls	5	1 (0.7)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	5.3 (0.9)
	Cases	5	1 (0.7)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	11.7 (1.9)
BDE 99	Controls	5	2 (1.3)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	161.6 (23.7)
	Cases	5	5 (3.6)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	32.7 (5.6)
BDE 100	Controls	5	2 (1.3)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	146.3 (21.5)
	Cases	5	4 (2.9)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	29.6 (5)
BDE 153	Controls	130	1 (0.7)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	150 (22)
	Cases	130	0	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)

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BDE 154	Controls	25	3 (2)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	125 (18.3)
	Cases	25	1 (0.7)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)	72 (11.8)

¹Aldrin, o,p'-DDT, α -endosulfan, β -endosulfan, trans-heptachlor epoxide, PCB 31, BDE congener 85 & 183, polybromobiphenyl 153, and $\alpha\beta$ -hexabromocyclododecane concentrations were below the LOD in all cases and controls.

²These POPS could not be distinguished during the measurement process.

Abbreviations: N, number of subjects; LOD, limit of detection.

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Table III. Associations between serum persistent organic pollutants and diminished ovarian reserve (defined as AFC < 7 and/or AMH ≤ 1.1 ng/ml) in the AROPE study (N = 138 cases and 151 controls)

Persistent organic pollutant	Concentration (ng/g lipid base) ²	Number of cases (N = 138)	Number of controls (N = 151)	Diminished ovarian reserve			
				Univariate logistic regression		Multivariate ¹ logistic regression	
				OR	CI	OR	CI
Organochlorine pesticides							
p,p'-DDE	≤ 22.39	29	51	Ref.	Ref.	Ref.	Ref.
	(22.39-40.74]	51	50	1.76	0.96 - 3.21	1.46	0.74 - 2.87
	> 40.74	58	50	1.96	1.08 - 3.55	1.72	0.88 - 3.37
p,p'-DDT	Continuous	138	151	1.40	1.13 - 1.74	1.39	1.10 - 1.77
	< LOD	52	51	Ref.	Ref.	Ref.	Ref.
	≥ LOD	86	100	1.19	0.73 - 1.92	1.13	0.66 - 1.94
cis-Heptachlor epoxide	≤ 1.71	53	50	Ref.	Ref.	Ref.	Ref.
	(1.71-2.42]	50	51	0.94	0.54 - 1.64	0.84	0.47 - 1.52
	> 2.42	35	50	0.65	0.36 - 1.15	0.64	0.35 - 1.18
HCB	Continuous	138	151	0.89	0.58 - 1.36	0.86	0.55 - 1.35
	≤ 6.43	38	51	Ref.	Ref.	Ref.	Ref.
	(6.43-8.42]	54	50	1.42	0.80 - 2.51	1.07	0.57 - 2.01
β-HCH	> 8.42	46	50	1.19	0.66 - 2.21	0.89	0.45 - 1.77
	Continuous	138	151	1.08	0.66 - 1.79	0.85	0.47 - 1.53
	≤ 3.62	49	51	Ref.	Ref.	Ref.	Ref.
	(3.62-5.77]	53	50	1.08	0.62 - 1.88	0.77	0.42 - 1.42
	> 5.77	36	50	0.72	0.40 - 1.29	0.43	0.21 - 0.84
	Continuous	138	151	0.81	0.62 - 1.07	0.63	0.44 - 0.89
Polychlorinated biphenyls							
PCB 28	≤ 0.41	41	49	Ref.	Ref.	Ref.	Ref.
	(0.41-0.60]	45	51	0.97	0.46 - 2.04	0.97	0.44 - 2.12
	> 0.60	52	51	1.25	0.68 - 2.30	1.24	0.64 - 2.41
PCB 74	Continuous	138	151	1.05	0.77 - 1.43	1.00	0.71 - 1.39
	≤ 1.65	39	51	Ref.	Ref.	Ref.	Ref.
	(1.65-2.52]	48	49	1.21	0.68 - 2.15	1.00	0.53 - 1.89
PCB 99	> 2.52	51	51	1.31	0.74 - 2.31	0.91	0.47 - 1.76
	Continuous	138	151	1.02	0.78 - 1.32	0.84	0.61 - 1.15
	≤ 1.82	35	51	Ref.	Ref.	Ref.	Ref.
PCB 118	(1.82-2.69]	43	50	1.29	0.71 - 2.35	1.24	0.65 - 2.39
	> 2.69	60	50	1.73	0.97 - 3.07	1.52	0.80 - 2.90
	Continuous	138	151	1.20	0.87 - 1.65	1.04	0.72 - 1.50
PCB 138+163 ³	≤ 3.45	33	51	Ref.	Ref.	Ref.	Ref.
	(3.45-5.50]	55	50	1.67	0.93 - 2.99	1.43	0.76 - 2.70
	> 5.50	50	50	1.49	0.83 - 2.67	1.17	0.60 - 2.29
	Continuous	138	151	1.10	0.81 - 1.51	0.94	0.65 - 1.37
	≤ 3.71	33	51	Ref.	Ref.	Ref.	Ref.
	(3.71-6.44]	42	50	1.27	0.70 - 2.32	1.01	0.50 - 2.04

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	> 6.44	63	50	1.87	1.05 - 3.32	1.27	0.57 - 2.81
	Continuous	138	151	1.26	0.97 - 1.63	0.97	0.65 - 1.43
PCB 153	≤ 16.36	35	51	Ref.	Ref.	Ref.	Ref.
	(16.36-26.21]	50	50	1.43	0.80 - 2.56	1.09	0.56 - 2.16
	> 26.21	53	50	1.48	0.83 - 2.65	1.01	0.48 - 2.12
	Continuous	138	151	1.29	0.94 - 1.77	1.00	0.65 - 1.55
PCB 170	≤ 11.34	35	51	Ref.	Ref.	Ref.	Ref.
	(11.34-16.43]	33	50	0.94	0.51 - 1.75	0.75	0.38 - 1.49
	> 16.43	70	50	1.96	1.12 - 3.44	1.56	0.78 - 3.12
	Continuous	138	151	1.27	0.92 - 1.75	1.00	0.66 - 1.51
PCB 180	≤ 10.85	32	51	Ref.	Ref.	Ref.	Ref.
	(10.85-18.91]	45	50	1.41	0.77 - 2.56	1.10	0.54 - 2.21
	> 18.91	61	50	1.87	1.05 - 3.33	1.20	0.54 - 2.67
	Continuous	138	151	1.30	0.99 - 1.69	1.01	0.68 - 1.51
PCB 183	≤ 0.94	30	51	Ref.	Ref.	Ref.	Ref.
	(0.94-1.52]	51	51	1.70	0.94 - 3.09	1.43	0.73 - 2.81
	> 1.52	57	49	1.86	1.03 - 3.36	1.43	0.69 - 2.96
	Continuous	138	151	1.42	1.04 - 1.94	1.23	0.81 - 1.85
PCB 187	≤ 2.06	40	52	Ref.	Ref.	Ref.	Ref.
	(2.06-3.54]	41	52	1.17	0.64 - 2.13	0.99	0.49 - 1.97
	> 3.54	57	47	1.74	0.98 - 3.07	1.38	0.68 - 2.77
	Continuous	138	151	1.23	0.96 - 1.57	1.07	0.78 - 1.47
PCB 194	≤ 1.23	30	52	Ref.	Ref.	Ref.	Ref.
	(1.23-2.38]	59	49	1.90	1.05 - 3.43	1.40	0.69 - 2.85
	> 2.38	49	50	1.67	0.92 - 3.03	0.87	0.37 - 2.05
	Continuous	138	151	1.34	1.05 - 1.71	1.09	0.74 - 1.60
PCB 196+203 ³	≤ 1.12	29	51	Ref.	Ref.	Ref.	Ref.
	(1.12-1.89]	53	51	1.83	1.00 - 3.33	1.50	0.73 - 3.09
	> 1.89	56	49	1.89	1.04 - 3.43	1.42	0.63 - 3.22
	Continuous	138	151	1.37	1.05 - 1.80	1.16	0.78 - 1.72

mass index, centre, education level, and season of inclusion.

nsformed when treated as continuous variables.

be distinguished during the measurement process.

er of subjects; OR, odds ratio; CI, confidence interval; Ref, reference.

Table IV. Posterior inclusion probabilities for group inclusion and conditional inclusion in the Bayesian Kernel Machine Regression model (N = 137 cases and 151 controls)

Persistent organic pollutant	Group	Diminished ovarian reserve	
		Group PIP (%)	Conditional PIP (%)
p,p'-DDE	1	96.9	6.8
cis-Heptachlor epoxide	1	96.9	1.0
HCB	1	96.9	1.0
β -HCH	1	96.9	91.4
PCB 28	2	31.8	6.9
PCB 74	2	31.8	3.0
PCB 99	2	31.8	17.6
PCB 118	2	31.8	6.0
PCB 138+163	2	31.8	6.0
PCB 153	2	31.8	9.2
PCB 170	2	31.8	7.1
PCB 180	2	31.8	9.3
PCB 183	2	31.8	11.5
PCB 187	2	31.8	7.2
PCB 194	2	31.8	7.0
PCB 196+203	2	31.8	9.1

Abbreviations: PIP, posterior inclusion probability.

Detailed Persistent Organic Pollutant (POP) measurement methods

Reagents and chemicals

Acetone, dichloromethane (DCM), and n-hexane (PESTIPUR - for pesticide analysis) were purchased from CARLO ERBA Reagents S.A.S (Val de Reuil, France). Absolute methanol (MeOH) (ULC/MS - CC/SFC) was purchased from Biosolve Chimie SARL (Dieuze, France). Formic acid (99%, for analysis) was purchased from CARLO ERBA Reagents S.A.S (Val de Reuil, France). Strata C18-E cartridges (6 mL, 500 mg) were purchased from Phenomenex France (Le Pecq, France). Certified standards of aldrin, o,p'-DDE, o,p'-DDT, p,p'-DDE, p,p'-DDT, dieldrin, α -endosulfan, β -endosulfan, α -HCH, β -HCH, γ -HCH (lindane), heptachlor, cis-heptachlor epoxide, trans-heptachlor epoxide, and hexachlorobenzene (HCB) were purchased from LGC Labor GmbH (Augsburg, Germany). The purity of the standards was above 97%. Individual standard stock solutions (1 g/L) were prepared in acetone by accurately weighing 25 mg (\pm 0.1 mg) of each standard using a Sartorius Cubis MSE 225P semi-micro balance (Sartorius AG, Göttingen, Germany) into 25-mL volumetric flasks and stored at -18°C . A cyclohexane mixture (10 mg/L) of eight PCBs (PCB 28, 31, 52, 101, 118, 138, 153, and 180) and isooctane solutions (10 mg/L) of PCB 74, 99, 170, 183, 187, 194, and 203 were purchased from LGC Labor GmbH (Augsburg, Germany). Nonane/toluene (10%) solutions (50 mg/L) of PBB 153 and BDE 28, 47, 66, 85, 99, 100, 153, 154, and 183 and a toluene mixture (10 mg/L) of three HBCD isomers (α -, β - and γ -HBCD) were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Intermediate and spiking solutions were prepared in acetone by appropriate dilution of individual standard stock solutions and commercial solutions.

A toluene/nonane (25.2%) mixture (2 mg/L) of seven $^{13}\text{C}_{12}$ -PBDEs (BDE 28, 47, 99, 100, 153, 154, and 183), a nonane/toluene (7%) mixture (5 mg/L) of seven $^{13}\text{C}_{12}$ -PCBs (PCB 28, 52, 101, 118, 138, 153, and 180), and a nonane/toluene (10%) solution (50 mg/L) of PCB 194 $^{13}\text{C}_{12}$ were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Acetone solutions (100 mg/L) of p,p'-DDE D_8 , p,p'-DDT $^{13}\text{C}_{12}$, and α -endosulfan D_4 were purchased from LGC Labor GmbH (Augsburg, Germany). Nonane solutions (100 mg/L) of aldrin $^{13}\text{C}_{12}$, o,p'-DDT $^{13}\text{C}_{12}$, dieldrin $^{13}\text{C}_{12}$, β -endosulfan $^{13}\text{C}_9$, HCB $^{13}\text{C}_6$, γ -HCH $^{13}\text{C}_6$, and cis-

heptachlor epoxide $^{13}\text{C}_{10}$ and a nonane solution (50 mg/L) of β -HCH $^{13}\text{C}_6$ were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA).

The standard reference materials SRM 1957 (Organic Contaminants in Non-Fortified Human Serum (Freeze-Dried)) and SRM 1958 (Organic Contaminants in Fortified Human Serum (Freeze-Dried)) were purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

Sample pre-treatment (protein denaturation)

Two milliliters of serum were placed in a 10-mL glass tube previously rinsed with methanol. After adding 2 mL formic acid and the labeled internal standards (ISTDs), the serum sample was subsequently sonicated for 30 minutes making sure that the temperature of the bath did not exceed 25°C. After a return to room temperature, 4 mL ultra-pure water were added to the acidified sample to reach a final volume of 8 mL.

Solid-phase extraction (SPE)

Extractions were performed using a Supelco Visiprep DL (disposable liner) vacuum manifold and a GAST vacuum pump. A Strata C18-E cartridge (6 mL, 500 mg) was successively conditioned with 20 mL hexane, 10 mL DCM, 5 mL MeOH, 5 mL of a 50% MeOH/50% ultra-pure water mixture, and 5 mL ultra-pure water. Then, the pre-treated serum sample (8 mL) was slowly loaded. The cartridge was washed with 10 mL ultra-pure water and subsequently completely dried for a minimum of 60 min. Analytes were eluted with 5 mL hexane. The organic extract was then stored in a freezer for a minimum of 2 hours, evaporated at 30°C under a nitrogen stream using a N-EVAP 111 Organomation Nitrogen Evaporator and adjusted to 500 μL of hexane prior to be transferred into a 2 mL amber glass vial and stored at -18°C prior to analysis by GC/MS/MS.

GC/MS/MS analysis

Analyses were performed using a 7890B GC system coupled to a 7010B GC/MS Triple Quad (Agilent Technologies, Santa Clara, California, United States) operated in electron impact ionization (EI) mode (70 eV). The GC system was equipped with a 7693 Autosampler and a multimode inlet (MMI) fitted with an empty (no wool) single taper ultra-inert glass liner. Calibration solutions and sample extracts were injected (2 μ L) in the splitless mode (purge flow to split vent: 40 mL/min at 2 min) with the following injector temperature program: 58°C (hold 0.1 min), ramp at 720°C/min to 320 °C (hold 5 min). Helium was used as the column carrier gas at a constant flow rate of 1.0 mL/min. Chromatographic separation was performed on a Rtx[®]-PCB capillary column (30 m length \times 0.25 mm I.D., 0.25- μ m film thickness) supplied by Restek Corporation (Bellefonte, Pennsylvania, United States) with the following oven temperature program: 58°C (hold 2 min), first ramp at 45°C/min to 200°C (hold 0 min), second ramp at 20°C/min to 320°C (hold 15 min to reach an analysis time of 26.2 min). The MSD transfer line, ion source, and quadrupole temperatures were set to 315, 270, and 150°C, respectively. The mass spectrometer (triple quadrupole) was operated in multiple reaction monitoring (MRM) mode. The two most intense and specific MRM transitions of each compound (quantifier and qualifier transitions) were monitored for identification, confirmation, and quantification. They were selected using the pesticides and environmental pollutants MRM database provided by Agilent Technologies for the compounds present in the database or following the usual procedure for others. Dwell times ranged between 15 and 200 ms in 16 time segments. Dwell times were thus fixed, taking into account the number of MRM transitions in each time segment and the width of the chromatographic peaks, such that each peak was represented by at least 10 data points from its beginning to the end. The analytical characteristics of the measured compounds are reported in Supplemental Material S2. MassHunter software (B.07.06) was used for instrument control, data acquisition, and quantification.

Quality assurance and quality control (QA/QC)

The limits of detection (LODs) were defined as the lowest concentration of a substance that can be distinguished from the absence of that substance. LODs were estimated from the replicate analysis of a blank sample. Limits of quantification (LOQs) were defined as the lowest concentration of a substance for which the relative standard deviation (RSD) of the raw signal ($N = 5$) was lower than or equal to 20%, the signal-to-noise ratio (S/N) was greater than or equal to 10, and the raw signal was greater than or equal to 5 times the signal of the blank sample.

Several labeled substances were selected to best cover the physical and chemical properties of the targeted analytes. Aldrin $^{13}\text{C}_{12}$, o,p'-DDT $^{13}\text{C}_{12}$, p,p'-DDE D_8 , p,p'-DDT $^{13}\text{C}_{12}$, dieldrin $^{13}\text{C}_{12}$, α -endosulfan D_4 , β -endosulfan $^{13}\text{C}_9$, HCB $^{13}\text{C}_6$, β -HCH $^{13}\text{C}_6$, γ -HCH $^{13}\text{C}_6$, cis-heptachlor epoxide $^{13}\text{C}_{10}$, $^{13}\text{C}_{12}$ -PCB 28, 52, 101, 118, 138, 153, 180 and 194, and $^{13}\text{C}_{12}$ -BDE 28, 47, 99, 100, 153, 154, and 183 were added prior to the protein denaturation step and used as internal standards (ISTDs). All compounds were quantified using the appropriate ISTD (Table S1) to compensate for the variability associated with the SPE-GC/MS/MS analysis from calibration curves generated for each compound by analyzing at least five different calibration samples. A quadratic fit (origin ignored, no weighting) was used to compensate for the nonlinearity of the instrument response over a wide working range.

Matrix interference can affect the detection of POPs in serum samples due to the presence of non-target compounds that may react with the target analytes or that may be similar to and co-elute with the target analytes. Two standard reference materials (SRMs) were used to control method accuracy and precision and identify possible interference due to the matrix: SRM 1957 (Organic Contaminants in Non-Fortified Human Serum (Freeze-Dried)) and SRM 1958 (Organic Contaminants in Fortified Human Serum (Freeze-Dried)). Certified or reference concentrations are provided for certain PBBs, PCBs, chlorinated pesticides, and PBDEs, depending on the standard reference material. Method accuracy and precision were assessed via replicate analysis of both SRMs during the analysis campaign. The arithmetic means of the measured concentrations were compared to reference or certified concentrations, and method precision was defined as the RSD of the replicates. The results are reported in Supplemental Material S3.

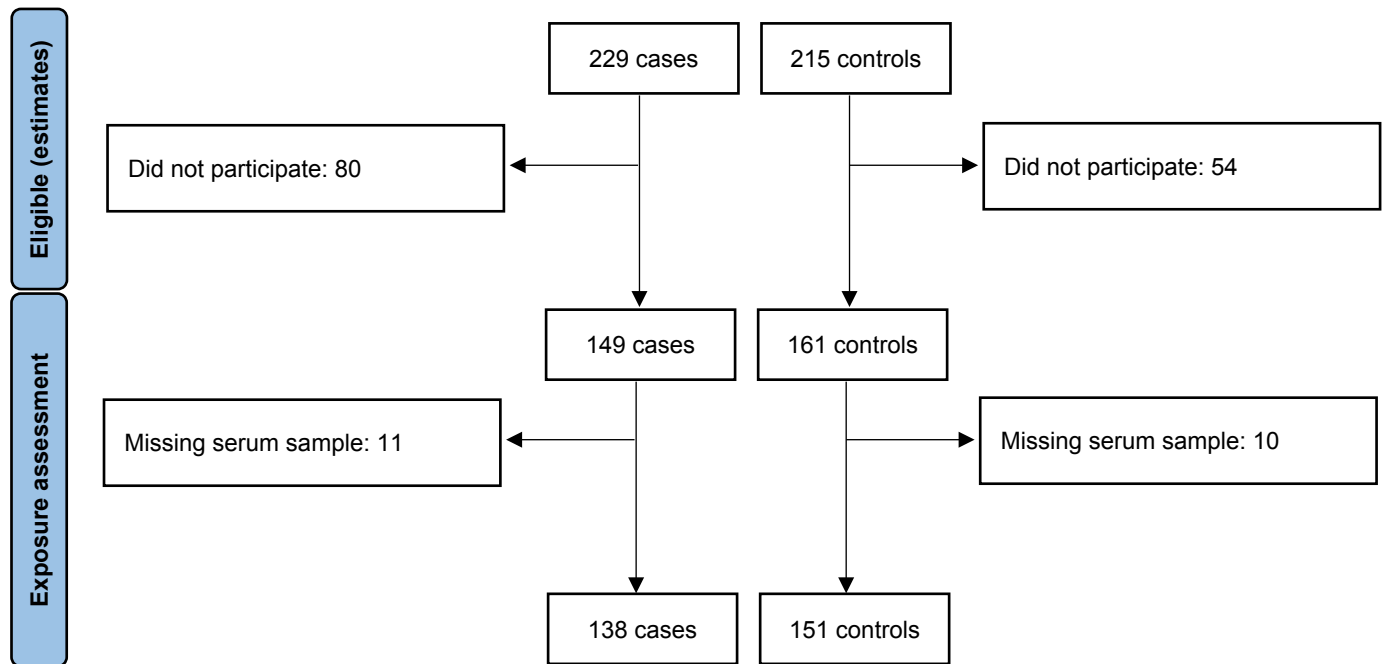
Each batch included: i) up to 51 samples (2 mL), ii) one procedural calibration blank sample and six to eight procedural calibration samples, depending on the substance prepared from ultra-pure water and analyzed as regular samples to assess whether contamination may have occurred during analysis and to generate quadratic calibration curves intended for quantification, respectively, iii) several procedural calibration samples prepared from commercial solutions provided by other suppliers and analyzed as regular samples to validate the preparation of the calibration solutions, iv) one procedural blank sample and two procedural QC samples (2-mL ultra-pure water non-spiked and spiked at the LOQ level and at an intermediate level) analyzed as regular samples every 21 samples maximum to assess whether contamination may have occurred during analysis, and to check for method accuracy and the stability of the detector response, respectively, and v) two samples of SRM 1957 and SRM 1958 (undiluted and diluted one tenth in ultra-pure water) analyzed as regular samples to check for method accuracy.

Positive values for each substance were confirmed by comparing retention times and MRM transition ratios ($\pm 30\%$) between calibration and serum samples. The data validation protocol included several conditions: i) the determination coefficient of the calibration curve had to be greater than 0.99, ii) the concentration of a substance measured in the procedural calibration samples had to be within $\pm 50\%$ of its theoretical concentration value at the LOQ level and $\pm 25\%$ at all other levels, iii) the response of a substance (ISTD response ratio) in the procedural blank samples had to be lower than 50% of that in the procedural calibration sample at the LOQ level, iv) the concentration of a substance measured in the procedural calibration samples prepared from commercial solutions provided by other suppliers had to be within $\pm 25\%$ of its theoretical concentration value, (v) the concentration of a substance measured in the procedural QC samples had to be within $\pm 50\%$ of its theoretical concentration value at the LOQ level and $\pm 25\%$ at all other levels, (vi) the concentration of a substance measured in the samples of SRM 1957 and 1958 had to be within $\pm 30\%$ of its certified or reference value, and vii) the concentration of a substance measured in the serum samples had to be within the method working range without exceeding

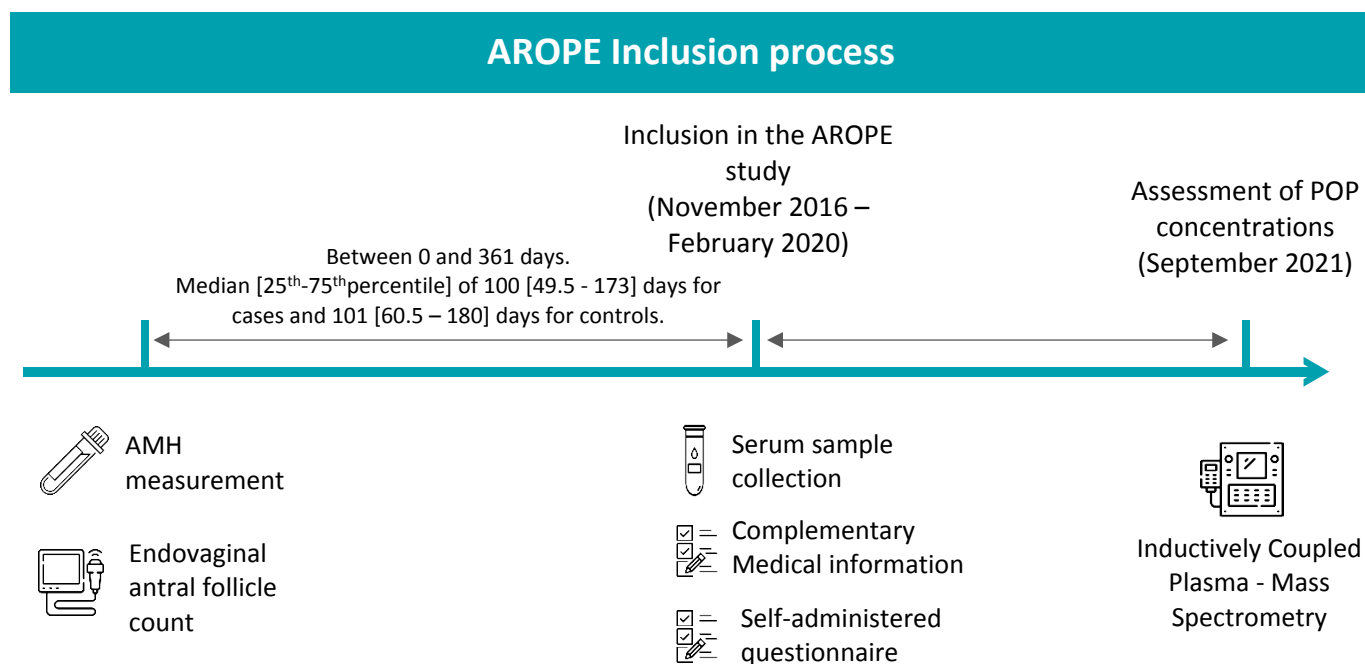
110% of the concentration of the most concentrated calibration samples. If all these conditions were not met, results were not validated, and the samples were reanalyzed if possible.

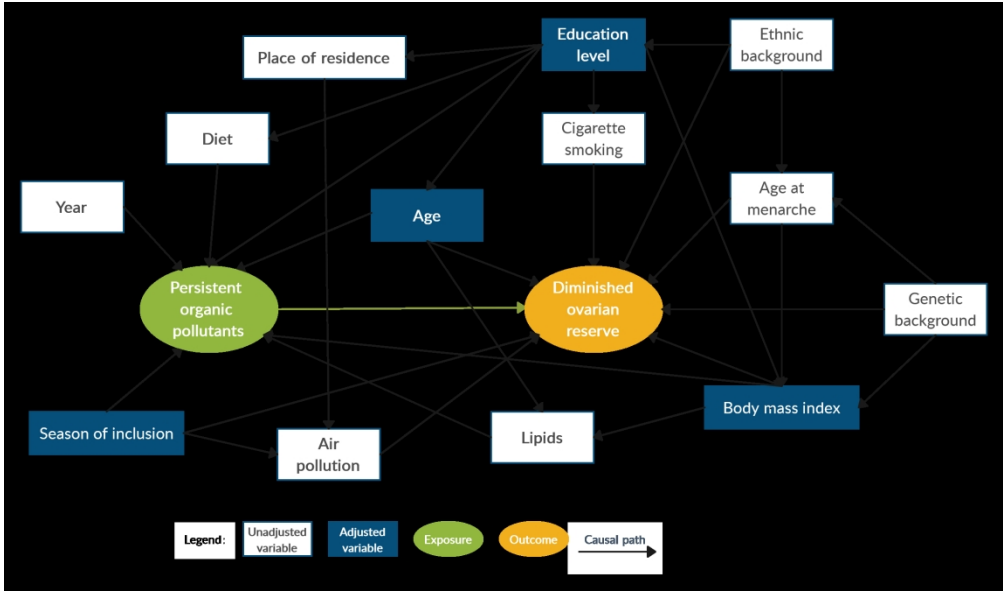
Multiple precautions were taken throughout the protocol to minimize procedural blank contamination. Glass materials, such as tubes, flasks, and syringes, were dedicated to this protocol and rigorously rinsed with dichloromethane and/or hexane prior to use and extraction cartridges rigorously conditioned. Despite these precautions, HCB and γ -HCH were regularly detected in the procedural blank samples (response (ISTD response ratio) higher than 50% of that in the procedural calibration sample at the LOQ level). If concentrations measured in a procedural blank sample exceeded 25% of those measured in a serum sample from the same batch, the concentrations reported here were adjusted for the procedural blank concentrations or the LOD and LOQ were increased (case of γ -HCH). Otherwise, the results were validated (case of HCB).

It should be noted that the laboratory successfully participated in inter-laboratory comparisons (ILCs) during the analysis campaign (external quality assessment schemes of the centre de toxicologie du Québec (CTQ): AMAP Ring Test for Persistent Organic Pollutants in Human Serum) and obtained Cofrac (French Committee for Accreditation) accreditation for this method, in accordance with the ISO/CEI 17025 standard (accreditation n°8-3557, www.cofrac.fr).

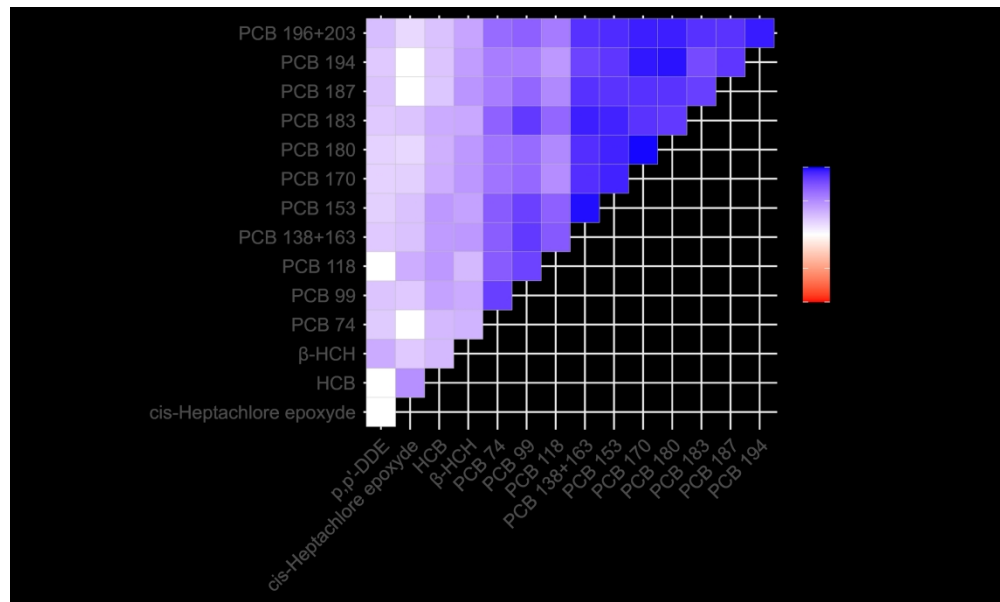
Supplementary Figure S1. AROPE study inclusion flowchart.

Supplementary Figure S2. AROPE study inclusion process.





825x486mm (72 x 72 DPI)



225x134mm (300 x 300 DPI)

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Supplementary Table SI. Analytical characteristics of measured compounds

Compound ^a	CAS number	Chemical family	ISTD	Time segment	t _R (min)	Quantifier MRM transition Precursor > Product (CE (V))	Qualifier MRM transition Precursor > Product (CE (V))	LOD ^b (µg/L)	LOQ ^b (µg/L)
Target compounds									
1	α-HCH	OCs	β-HCH ¹³ C ₆	2	8.8	216.9 > 181.0 (5)	180.9 > 145.0 (15)	0.0025	0.005
2	HCB	OCs	HCB ¹³ C ₆	2	8.8	283.8 > 213.9 (30)	281.8 > 211.9 (30)	0.0025	0.005
3	γ-HCH	OCs	γ-HCH ¹³ C ₆	2	9.1	216.9 > 181.0 (5)	181.0 > 145.0 (15)	0.0025	0.005
4	β-HCH	OCs	β-HCH ¹³ C ₆	3	9.4	216.9 > 181.1 (5)	181.0 > 145.0 (15)	0.0025	0.005
5	PCB 31	PCBs	PCB 28 ¹³ C ₁₂	3	9.6	256.0 > 186.0 (25)	258.0 > 186.0 (25)	0.0025	0.005
6	PCB 28	PCBs	PCB 28 ¹³ C ₁₂	3	9.6	256.0 > 186.0 (25)	258.0 > 186.0 (25)	0.0025	0.005
7	Heptachlor	OCs	cis-Heptachlor epoxide ¹³ C ₁₀	3	9.7	271.7 > 236.9 (15)	273.7 > 238.9 (15)	0.0025	0.005
8	PCB 52	PCBs	PCB 52 ¹³ C ₁₂	3	9.8	289.9 > 219.9 (25)	291.9 > 221.9 (25)	0.0025	0.005
9	Aldrin	OCs	Aldrin ¹³ C ₁₂	3	10.0	262.9 > 192.9 (35)	254.9 > 220.0 (20)	0.025	0.05
10	cis-Heptachlor epoxide	OCs	cis-Heptachlor epoxide ¹³ C ₁₀	4	10.5	352.8 > 262.9 (15)	354.8 > 264.9 (15)	0.005	0.01
11	PCB 74	PCBs	PCB 52 ¹³ C ₁₂	4	10.5	289.9 > 219.9 (25)	291.9 > 221.9 (25)	0.0025	0.005
12	trans-Heptachlor epoxide	OCs	cis-Heptachlor epoxide ¹³ C ₁₀	4	10.5	135.0 > 099.0 (15)	216.9 > 182.0 (20)	0.025	0.05
13	o,p'-DDE	OCs	p,p'-DDE D ₈	4	10.6	246.0 > 176.2 (30)	248.0 > 176.2 (30)	0.0025	0.005
14	PCB 101	PCBs	PCB 101 ¹³ C ₁₂	4	10.7	253.9 > 184.0 (35)	325.9 > 255.9 (30)	0.0025	0.005
15	PCB 99	PCBs	PCB 101 ¹³ C ₁₂	4	10.7	253.9 > 184.0 (40)	325.9 > 255.9 (25)	0.0025	0.005
16	α-Endosulfan	OCs	α-Endosulfan D ₄	5	10.9	194.9 > 159.0 (5)	194.9 > 125.0 (20)	0.025	0.05
17	p,p'-DDE	OCs	p,p'-DDE D ₈	5	10.9	246.1 > 176.2 (30)	315.8 > 246.0 (15)	0.01	0.02
18	Dieldrin	OCs	Dieldrin ¹³ C ₁₂	6	11.2	277.0 > 241.0 (5)	262.9 > 193.0 (35)	0.025	0.05
19	o,p'-DDT	OCs	o,p'-DDT ¹³ C ₁₂	7	11.3	235.0 > 165.2 (20)	237.0 > 165.2 (20)	0.025	0.05
20	PCB 118	PCBs	PCB 118 ¹³ C ₁₂	7	11.4	253.9 > 184.0 (35)	325.9 > 255.9 (30)	0.0025	0.005
21	BDE 28	PBDEs	BDE 28 ¹³ C ₁₂	7	11.5	246.0 > 139.0 (30)	406.0 > 246.0 (20)	0.0025	0.005
22	PCB 153	PCBs	PCB 153 ¹³ C ₁₂	7	11.5	359.9 > 289.9 (25)	287.9 > 217.9 (40)	0.0025	0.005
23	β-Endosulfan	OCs	β-Endosulfan ¹³ C ₉	8	11.7	206.9 > 172.0 (15)	194.9 > 158.9 (10)	0.025	0.05
24	p,p'-DDT	OCs	p,p'-DDT ¹³ C ₁₂	8	11.8	235.0 > 165.2 (20)	235.0 > 199.2 (15)	0.005	0.01
25	PCB 138 + PCB 163	PCBs	PCB 138 ¹³ C ₁₂	8	11.8	359.9 > 289.9 (30)	287.9 > 217.9 (40)	0.0025	0.005
26	PCB 187	PCBs	PCB 138 ¹³ C ₁₂	8	11.9	393.8 > 323.8 (30)	395.8 > 360.8 (15)	0.0025	0.005
27	PCB 183	PCBs	PCB 180 ¹³ C ₁₂	8	11.9	393.8 > 323.8 (30)	323.8 > 253.8 (40)	0.0025	0.005
28	PCB 180	PCBs	PCB 180 ¹³ C ₁₂	9	12.5	393.8 > 323.8 (30)	395.8 > 325.8 (30)	0.0025	0.005
29	BDE 47	PBDEs	BDE 47 ¹³ C ₁₂	10	12.8	325.8 > 216.8 (30)	485.7 > 325.7 (20)	0.005	0.01
30	α + β + γ-HBCD	HBCDs	BDE 47 ¹³ C ₁₂	10	12.9	238.9 > 159.1 (5)	320.9 > 159.1 (8)	2.5	5
31	PCB 170	PCBs	PCB 180 ¹³ C ₁₂	10	13.0	393.8 > 323.8 (30)	395.8 > 360.8 (15)	0.0025	0.005
32	PCB 196 + PCB 203	PCBs	PCB 194 ¹³ C ₁₂	10	13.0	427.8 > 357.8 (30)	429.8 > 359.8 (30)	0.0025	0.005
33	BDE 66	PBDEs	BDE 47 ¹³ C ₁₂	10	13.1	326.0 > 217.0 (30)	486.0 > 326.0 (20)	0.005	0.01
34	PCB 194	PCBs	PCB 194 ¹³ C ₁₂	11	13.9	427.8 > 357.8 (30)	429.8 > 394.8 (15)	0.0025	0.005
35	BDE 100	PBDEs	BDE 100 ¹³ C ₁₂	12	14.1	405.8 > 296.7 (35)	565.5 > 405.6 (20)	0.005	0.01
36	BDE 99	PBDEs	BDE 99 ¹³ C ₁₂	13	14.6	565.6 > 405.6 (20)	405.7 > 296.7 (35)	0.005	0.01
37	BDE 85	PBDEs	BDE 99 ¹³ C ₁₂	14	16.0	406.0 > 297.0 (35)	564.0 > 404.0 (25)	0.125	0.25
38	BDE 154	PBDEs	BDE 154 ¹³ C ₁₂	14	16.1	643.6 > 483.6 (25)	483.6 > 376.8 (35)	0.025	0.05
39	PBB 153	PBBs	BDE 154 ¹³ C ₁₂	14	16.4	467.6 > 307.6 (35)	307.7 > 147.8 (30)	0.025	0.05
40	BDE 153	PBDEs	BDE 153 ¹³ C ₁₂	15	17.3	483.7 > 323.6 (40)	643.6 > 483.6 (20)	0.125	0.25

	Compound ^a	CAS number	Chemical family	ISTD	Time segment	t _R (min)	Quantifier MRM transition Precursor > Product (CE (V))	Qualifier MRM transition Precursor > Product (CE (V))	LOD ^b (µg/L)	LOQ ^b (µg/L)	
41	BDE 183	207122-16-5	PBDEs	BDE 183 ¹³ C ₁₂	16	21.7	721.8 > 561.7 (20)	561.7 > 454.6 (35)	2.5	5	
	Labeled ISTDs										
a	HCB ¹³ C ₆	93952-14-8	OCs		2	8.8	289.8 > 219.8 (35)	-			
b	γ-HCH ¹³ C ₆	58-89-9	OCs		2	9.1	186.8 > 151.0 (15)	-			
c	β-HCH ¹³ C ₆	222966-68-9	OCs		3	9.4	224.8 > 189.0 (10)	-			
d	PCB 28 ¹³ C ₁₂	208263-76-7	PCBs		3	9.6	267.9 > 198.1 (30)	-			
e	PCB 52 ¹³ C ₁₂	208263-80-3	PCBs		3	9.8	301.9 > 232.1 (30)	-			
f	Aldrin ¹³ C ₁₂	n/a	OCs		3	10.0	268.0 > 198.0 (35)	-			
g	cis-Heptachlor epoxide ¹³ C ₁₀	1024-57-3	OCs		4	10.5	363.0 > 269.9 (15)	-			
h	PCB 101 ¹³ C ₁₂	104130-39-4	PCBs		4	10.7	265.9 > 196.0 (35)	-			
i	α-Endosulfan D ₄	203645-57-2	OCs		5	10.9	163.9 > 129.0 (15)	-			
j	p,p'-DDE D ₈	93952-19-3	OCs		5	10.9	254.1 > 184.1 (35)	-			
k	Dieldrin ¹³ C ₁₂	475274-96-5	OCs		6	11.2	284.8 > 213.9 (30)	-			
l	o,p'-DDT ¹³ C ₁₂	1396995-26-8	OCs		7	11.3	249.0 > 177.1 (28)	-			
m	PCB 118 ¹³ C ₁₂	104130-40-7	PCBs		7	11.4	335.9 > 266.0 (35)	-			
n	BDE 28 ¹³ C ₁₂	n/a	PBDEs		7	11.5	257.9 > 150.1 (35)	-			
o	PCB 153 ¹³ C ₁₂	185376-58-3	PCBs		7	11.5	371.8 > 302.0 (32)	-			
p	β-Endosulfan ¹³ C ₉	n/a	OCs		8	11.7	202.0 > 166.0 (10)	-			
q	p,p'-DDT ¹³ C ₁₂	104215-84-1	OCs		8	11.8	249.0 > 177.1 (25)	-			
r	PCB 138 ¹³ C ₁₂	208263-66-5	PCBs		8	11.8	301.9 > 232.0 (40)	-			
s	PCB 180 ¹³ C ₁₂	160901-82-6	PCBs		9	12.5	405.8 > 336.0 (35)	-			
t	BDE 47 ¹³ C ₁₂	n/a	PBDEs		10	12.8	337.9 > 230.0 (32)	-			
u	PCB 194 ¹³ C ₁₂	208263-74-5	PCBs		11	13.9	441.8 > 371.9 (35)	-			
v	BDE 100 ¹³ C ₁₂	n/a	PBDEs		12	14.1	575.7 > 415.9 (25)	-			
w	BDE 99 ¹³ C ₁₂	n/a	PBDEs		13	14.6	415.8 > 307.9 (38)	-			
x	BDE 154 ¹³ C ₁₂	n/a	PBDEs		14	16.1	655.7 > 495.8 (25)	-			
y	BDE 153 ¹³ C ₁₂	n/a	PBDEs		15	17.3	655.7 > 495.8 (25)	-			
z	BDE 183 ¹³ C ₁₂	n/a	PBDEs		16	21.7	573.9 > 465.8 (40)	-			

^a Compounds listed in order of retention times; ^b for a 2-mL sample of serum

Supplementary Table SII. POP concentrations (ng/L) in the standard reference materials (SRMs) 1957 and 1958 (N = 7)

Compound ^a	LOD (ng/L)	LOQ (ng/L)	POP concentrations (ng/L) in SRM 1957 (n = 7)			POP concentrations (ng/L) in SRM 1958 (n = 7)		
			Measured (RSD %)	Reported	Measured/ reported (%)	Measured (RSD %)	Reported	Measured/ reported (%)
α-HCH	2.5	5.0	< 5.0	n.r.	-	224 (10)	260 ^c	86
HCB	2.5	5.0	31.4 (15)	29.7 ^b	106	393 (15)	442 ^b	89
γ-HCH	2.5	5.0	< 5.0	n.r.	-	231 (9)	315 ^c	73
β-HCH	2.5	5.0	26.6 (8)	31.3 ^c	85	243 (4)	278 ^c	87
PCB 28	2.5	5.0	7.44 (20)	8.6 ^c	87	403 (3)	402 ^b	100
PCB 52	2.5	5.0	< 5.0	n.r.	-	416 (4)	401 ^b	104
Aldrin	25	50	< 50	n.r.	-	373 (5)	n.r.	-
cis-Heptachlor epoxide	5.0	10	14.8 (6)	n.r.	-	392 (5)	n.r.	-
PCB 74	2.5	5.0	11.8 (5)	13.8 ^c	86	394 (3)	414 ^b	95
o,p'-DDE	2.5	5.0	< 5.0	n.r.	-	460 (6)	450 ^b	102
PCB 101	2.5	5.0	< 5.0	n.r.	-	385 (4)	409 ^b	94
PCB 99	2.5	5.0	10.1 (11)	11.6 ^c	87	373 (4)	385 ^b	97
p,p'-DDE	10	20	928 (3)	921 ^b	101	1280 (4)	1250 ^b	102
Dieldrin	25	50	27.6 (19)	n.r.	-	483 (7)	n.r.	-
o,p'-DDT	25	50	< 50	n.r.	-	253 (3)	313 ^b	81
PCB 118	2.5	5.0	15.8 (7)	18.9 ^b	84	407 (5)	412 ^b	99
BDE 28	2.5	5.0	17.6 (21)	20.0 ^c	88	442 (15)	462 ^b	96
PCB 153	2.5	5.0	59.5 (4)	58.2 ^b	102	444 (5)	457 ^b	97
p,p'-DDT	5.0	10	< 10	n.r.	-	145 (4)	293 ^b	49
PCB 138 + PCB 163	2.5	5.0	43.4 (3)	36.9 ^b	118	451 (8)	473 ^b	95
PCB 187	2.5	5.0	12.6 (8)	15.5 ^b	82	357 (8)	411 ^b	87
PCB 183	2.5	5.0	4.49 (8)	5.77 ^c	78	355 (7)	407 ^b	87
PCB 180	2.5	5.0	49.1 (3)	54.5 ^b	90	431 (4)	459 ^b	94
BDE 47	5.0	10	237 (8)	268 ^b	88	577 (6)	651 ^b	89
PCB 170	2.5	5.0	14.3 (11)	16.2 ^b	88	395 (12)	422 ^b	94
PCB 196 + PCB 203	2.5	5.0	10.1 (6)	10.9 ^c	93	726 (9)	795 ^b	91
BDE 66	5.0	10	< 10	6.7 ^c	-	388 (7)	440 ^b	88
PCB 194	2.5	5.0	9.10 (6)	11.9 ^b	76	349 (8)	387 ^b	90
BDE 100	5.0	10	43.9 (26)	49.7 ^b	88	375 (7)	475 ^b	79
BDE 99	5.0	10	69.0 (28)	76.0 ^b	91	404 (7)	492 ^b	82
BDE 85	125	250	< 250	8.2 ^c	-	493 (14)	475 ^b	104
BDE 154	25	50	< 50	7.0 ^c	-	270 (6)	441 ^b	61
PBB 153	25	50	< 50	15.5 ^b	-	421 (10)	421 ^b	100
BDE 153	125	250	< 250	61.0 ^b	-	387 (6)	455 ^b	85
BDE 183	2500	5000	< 5000	n.r.	-	< 5000	453 ^b	-

n.r., not reported; ^a Compounds listed in order of retention times; ^b Certified concentration from the certificate of analysis of the Standard Reference Material; ^c Reference concentration from the certificate of analysis of the Standard Reference Material.

Supplementary Table SIII. Sensitivity analysis – Associations between serum persistent organic pollutants and diminished ovarian reserve in the AROPE study – Cases = AFC < 7 & AMH ≤ 1.1 ng/ml (N = 51 cases & 151 controls)

Persistent organic pollutant	Concentration (ng/g lipid base) ²	Number of cases (N = 51)	Number of controls (N = 151)	Diminished Ovarian Reserve Multivariate ¹ logistic regression	
				OR	CI
Organochlorine pesticides					
p,p'-DDE	≤ 22.39	10	51	Ref.	Ref.
]22.39-40.74]	21	50	1.45	0.56 - 3.73
	> 40.74	20	50	1.47	0.55 - 3.92
p,p'-DDT	Continuous	51	151	1.44	1.05 - 1.98
	< LOD	21	51	Ref.	Ref.
	≥ LOD	30	100	0.94	0.43 - 2.04
cis-Heptachlore epoxyde	≤ 1.71	17	51	Ref.	Ref.
]1.71-2.42]	17	50	0.72	0.30 - 1.74
	> 2.42	11	51	0.82	0.34 - 1.97
HCB	Continuous	51	151	0.97	0.50 - 1.88
	≤ 6.43	11	51	Ref.	Ref.
]6.43-8.42]	16	50	1.04	0.39 - 2.78
β-HCH	> 8.42	24	50	1.54	0.58 - 4.15
	Continuous	51	151	1.60	0.69 - 3.70
	≤ 3.62	15	51	Ref.	Ref.
]3.62-5.77]	22	50	1.11	0.47 - 2.61
	> 5.77	14	50	0.48	0.18 - 1.31
	Continuous	51	151	0.58	0.33 - 1.00
Polychlorinated biphenyls					
PCB 28	≤ 0.41	15	49	Ref.	Ref.
]0.41-0.60]	14	51	0.75	0.21 - 2.76
	> 0.60	22	51	1.45	0.57 - 3.70
PCB 74	Continuous	51	151	0.94	0.58 - 1.52
	≤ 1.65	11	51	Ref.	Ref.
]1.65-2.52]	18	49	1.16	0.45 - 3.01
PCB 99	> 2.52	22	51	1.32	0.50 - 3.44
	Continuous	51	151	1.04	0.67 - 1.61
	≤ 1.82	12	51	Ref.	Ref.
PCB 118]1.82-2.69]	16	50	1.26	0.49 - 3.23
	> 2.69	23	50	1.80	0.72 - 4.47
	Continuous	51	151	1.35	0.80 - 2.26
PCB 138+163 ³	≤ 3.45	8	51	Ref.	Ref.
]3.45-5.50]	21	50	2.73	0.97 - 7.68
	> 5.50	22	50	2.60	0.91 - 7.39
	Continuous	51	151	1.21	0.72 - 2.03
	≤ 3.71	12	51	Ref.	Ref.
]3.71-6.44]	14	50	1.07	0.40 - 2.86
	> 6.44	25	50	1.52	0.50 - 4.59

PCB 153	Continuous	51	151	1.19	0.69 - 2.07
	≤ 16.36	10	51	Ref.	Ref.
]16.36-26.21]	18	50	1.64	0.60 - 4.49
	> 26.21	23	50	1.87	0.64 - 5.44
PCB 170	Continuous	51	151	1.33	0.73 - 2.42
	≤ 11.34	11	51	Ref.	Ref.
]11.34-16.43]	11	50	0.84	0.30 - 2.34
	> 16.43	29	50	2.29	0.85 - 6.17
PCB 180	Continuous	51	151	1.22	0.69 - 2.15
	≤ 10.85	8	51	Ref.	Ref.
]10.85-18.91]	19	50	2.13	0.73 - 6.19
	> 18.91	24	50	2.33	0.68 - 8.03
PCB 183	Continuous	51	151	1.36	0.77 - 2.40
	≤ 0.94	10	51	Ref.	Ref.
]0.94-1.52]	18	51	1.54	0.58 - 4.09
	> 1.52	23	49	1.78	0.62 - 5.05
PCB 187	Continuous	51	151	1.41	0.80 - 2.48
	≤ 2.06	15	52	Ref.	Ref.
]2.06-3.54]	12	52	0.84	0.29 - 2.41
	> 3.54	24	47	2.09	0.78 - 5.62
PCB 194	Continuous	51	151	1.40	0.91 - 2.15
	≤ 1.23	10	52	Ref.	Ref.
]1.23-2.38]	19	49	1.46	0.52 - 4.08
	> 2.38	22	50	1.53	0.47 - 4.96
PCB 196+203 ³	Continuous	51	151	1.41	0.83 - 2.40
	≤ 1.12	9	51	Ref.	Ref.
]1.12-1.89]	18	51	1.80	0.62 - 5.20
	> 1.89	24	49	2.36	0.73 - 7.67
	Continuous	51	151	1.49	0.86 - 2.58

¹Adjusted for age, body-mass index, centre, education level, and season of inclusion.

²Log₂-transformed. Continuous analyses were only performed when linearity was respected.

³These POPS could not be distinguished during the measurement process.

Abbreviations: OR, odds ratio; CI, confidence interval; Ref, reference.

Supplementary Table SIV. Sensitivity analysis – Associations between serum persistent organic pollutants and diminished ovarian reserve in the AROPE study – Cases = AFC < 7 (N = 55 cases & 151 controls)

Persistent organic pollutant	Concentration (ng/g lipid base) ²	Number of cases (N = 55)	Number of controls (N = 151)	Diminished Ovarian Reserve Multivariate ¹ logistic regression	
				OR	CI
Organochlorine pesticides					
p,p'-DDE	≤ 22.39	11	51	Ref.	Ref.
]22.39-40.74]	23	50	1.49	0.60 - 3.68
	> 40.74	21	50	1.42	0.55 - 3.67
p,p'-DDT	Continuous	55	151	1.43	1.05 - 1.94
	< LOD	22	51	Ref.	Ref.
	≥ LOD	33	100	0.93	0.43 - 1.98
cis-Heptachlore epoxyde	≤ 1.71	18	50	Ref.	Ref.
]1.71-2.42]	18	51	0.77	0.33 - 1.81
	> 2.42	19	50	0.92	0.40 - 2.11
HCB	Continuous	55	151	1.17	0.47 - 2.92
	≤ 6.43	11	51	Ref.	Ref.
]6.43-8.42]	19	50	1.28	0.49 - 3.32
β-HCH	> 8.42	25	50	1.64	0.62 - 4.33
	Continuous	55	151	1.51	0.67 - 3.40
	≤ 3.62	15	51	Ref.	Ref.
Polychlorinated biphenyls]3.62-5.77]	26	50	1.35	0.59 - 3.10
	> 5.77	14	50	0.47	0.18 - 1.26
	Continuous	55	151	0.57	0.34 - 0.98
PCB 28	≤ 0.41	17	49	Ref.	Ref.
]0.41-0.60]	15	51	0.76	0.22 - 2.62
	> 0.60	23	51	1.36	0.55 - 3.38
	Continuous	55	151	0.94	0.58 - 1.52
PCB 74	≤ 1.65	12	51	Ref.	Ref.
]1.65-2.52]	20	49	1.18	0.47 - 2.94
	> 2.52	23	51	1.26	0.50 - 3.20
PCB 99	Continuous	55	151	0.98	0.54 - 1.81
	≤ 1.82	13	51	Ref.	Ref.
]1.82-2.69]	18	50	1.26	0.52 - 3.06
PCB 118	> 2.69	24	50	1.71	0.71 - 4.13
	Continuous	55	151	1.46	0.71 - 3.00
	≤ 3.45	8	51	Ref.	Ref.
PCB 138+163 ³]3.45-5.50]	24	50	2.98	1.09 - 8.11
	> 5.50	23	50	2.54	0.91 - 7.07
	Continuous	55	151	1.18	0.72 - 1.96
	≤ 3.71	13	51	Ref.	Ref.
]3.71-6.44]	16	50	1.07	0.42 - 2.73
	> 6.44	26	50	1.31	0.45 - 3.82

PCB 153	Continuous	55	151	1.08	0.63 - 1.86
	≤ 16.36	10	51	Ref.	Ref.
]16.36-26.21]	22	50	1.94	0.74 - 5.11
	> 26.21	23	50	1.72	0.60 - 4.93
PCB 170	Continuous	55	151	1.21	0.67 - 2.16
	≤ 11.34	11	51	Ref.	Ref.
]11.34-16.43]	14	50	1.07	0.40 - 2.82
	> 16.43	30	50	2.24	0.85 - 5.90
PCB 180	Continuous	55	151	1.13	0.65 - 1.95
	≤ 10.85	9	51	Ref.	Ref.
]10.85-18.91]	22	50	2.00	0.73 - 5.49
	> 18.91	24	50	1.76	0.54 - 5.72
PCB 183	Continuous	55	151	1.22	0.70 - 2.13
	≤ 0.94	10	51	Ref.	Ref.
]0.94-1.52]	22	51	1.85	0.72 - 4.74
	> 1.52	23	49	1.67	0.60 - 4.65
PCB 187	Continuous	55	151	1.56	0.70 - 3.47
	≤ 2.06	16	52	Ref.	Ref.
]2.06-3.54]	14	52	0.89	0.33 - 2.40
	> 3.54	25	47	1.91	0.74 - 4.94
PCB 194	Continuous	55	151	1.47	0.80 - 2.70
	≤ 1.23	11	52	Ref.	Ref.
]1.23-2.38]	22	49	1.49	0.56 - 3.98
	> 2.38	22	50	1.28	0.41 - 4.00
PCB 196+203 ³	Continuous	55	151	1.46	0.69 - 3.09
	≤ 1.12	9	51	Ref.	Ref.
]1.12-1.89]	21	51	2.03	0.72 - 5.70
	> 1.89	25	49	2.41	0.76 - 7.68
	Continuous	55	151	1.55	0.71 - 3.36

¹Adjusted for age, body mass-index, centre, education level, and season of inclusion.

²Log2-transformed. Continuous analyses were only performed when linearity was respected.

³These POPs could not be distinguished during the measurement process.

Abbreviations: OR, odds ratio; CI, confidence interval; Ref, reference.

Supplementary Table SV. Sensitivity analysis – Associations between serum persistent organic pollutants and diminished ovarian reserve in the AROPE study – Cases = AMH \leq 1.1 ng/ml (N = 134 cases & 151 controls)

Persistent organic pollutant	Concentration (ng/g lipid base) ²	Number of cases (N = 134)	Number of controls (N = 151)	Diminished Ovarian Reserve Multivariate ¹ logistic regression		
				OR	CI	
Organochlorine pesticides						
p,p'-DDE	\leq 22.39	28	51	Ref.	Ref.	
]22.39-40.74]	49	50	1.53	0.77 - 3.04	
	> 40.74	57	50	1.90	0.96 - 3.76	
	Continuous	134	151	1.39	1.09 - 1.77	
p,p'-DDT	< LOD	51	51	Ref.	Ref.	
	\geq LOD	83	100	1.14	0.67 - 1.96	
cis-Heptachlore epoxyde	\leq 1.71	52	50	Ref.	Ref.	
]1.71-2.42]	49	51	0.84	0.46 - 1.53	
	> 2.42	33	50	0.63	0.33 - 1.17	
	Continuous	134	151	0.82	0.52 - 1.30	
HCB	\leq 6.43	38	51	Ref.	Ref.	
]6.43-8.42]	51	50	0.82	0.52 - 1.29	
	> 8.42	45	50	1.03	0.54 - 1.94	
β -HCH	Continuous	134	151	0.92	0.46 - 1.84	
	\leq 3.62	49	51	Ref.	Ref.	
]3.62-5.77]	49	50	0.74	0.40 - 1.36	
	> 5.77	36	50	0.47	0.24 - 0.93	
Continuous		134	151	0.63	0.44 - 0.89	
	Polychlorinated biphenyls					
	PCB 28	\leq 0.41	39	49	Ref.	Ref.
]0.41-0.60]	44	51	0.99	0.44 - 2.25
> 0.60		51	51	1.32	0.66 - 2.63	
Continuous		134	151	1.02	0.72 - 1.43	
PCB 74	\leq 1.65	38	51	Ref.	Ref.	
]1.65-2.52]	46	49	0.99	0.52 - 1.89	
	> 2.52	50	51	0.94	0.48 - 1.83	
Continuous		134	151	0.85	0.62 - 1.17	
	PCB 99	\leq 1.82	34	51	Ref.	Ref.
]1.82-2.69]	41	50	1.26	0.65 - 2.44
> 2.69		59	50	1.62	0.84 - 3.12	
Continuous		134	151	1.04	0.72 - 1.51	
	PCB 118	\leq 3.45	33	51	Ref.	Ref.
]3.45-5.50]	52	50	1.43	0.75 - 2.71
> 5.50		49	50	1.26	0.64 - 2.49	
Continuous		134	151	0.95	0.65 - 1.38	
	PCB 138+163 ³	\leq 3.71	32	51	Ref.	Ref.
]3.71-6.44]	40	50	1.09	0.54 - 2.20

	> 6.44	62	50	1.51	0.68 - 3.35
PCB 153	Continuous	134	151	0.99	0.67 - 1.48
	≤ 16.36	35	51	Ref.	Ref.
]16.36-26.21]	46	50	1.05	0.53 - 2.09
	> 26.21	53	50	1.11	0.52 - 2.34
PCB 170	Continuous	134	151	1.04	0.67 - 1.60
	≤ 11.34	35	51	Ref.	Ref.
]11.34-16.43]	30	50	0.70	0.35 - 1.40
	> 16.43	69	50	1.68	0.83 - 3.39
PCB 180	Continuous	134	151	1.03	0.68 - 1.56
	≤ 10.85	31	51	Ref.	Ref.
]10.85-18.91]	42	50	1.11	0.55 - 2.26
	> 18.91	61	50	1.43	0.64 - 3.19
PCB 183	Continuous	134	151	1.04	0.69 - 1.56
	≤ 0.94	30	51	Ref.	Ref.
]0.94-1.52]	47	51	1.36	0.69 - 2.69
	> 1.52	57	49	1.57	0.75 - 3.27
PCB 187	Continuous	134	151	1.23	0.82 - 1.84
	≤ 2.06	39	52	Ref.	Ref.
]2.06-3.54]	39	52	1.00	0.49 - 2.03
	> 3.54	56	47	1.49	0.73 - 3.04
PCB 194	Continuous	134	151	1.09	0.79 - 1.50
	≤ 1.23	29	52	Ref.	Ref.
]1.23-2.38]	56	49	1.47	0.72 - 3.00
	> 2.38	49	50	1.02	0.43 - 2.40
PCB 196+203 ³	Continuous	134	151	1.11	0.75 - 1.64
	≤ 1.12	29	51	Ref.	Ref.
]1.12-1.89]	50	51	1.46	0.70 - 3.01
	> 1.89	55	49	1.51	0.66 - 3.45
	Continuous	134	151	1.19	0.80 - 1.78

¹Adjusted for age, body-mass index, centre, education level, and season of inclusion.

²Log2-transformed. Continuous analyses were only performed when linearity was respected.

³These POPS could not be distinguished during the measurement process.

Abbreviations: OR, odds ratio; CI, confidence interval; Ref, reference.

Supplementary Table SVI. Sensitivity analysis – Associations between serum persistent organic pollutants and diminished ovarian reserve in the AROPE study – Cases = AFC < 5 and/or AMH ≤ 0.7 ng/ml (N = 90 cases & 151 controls)

Persistent organic pollutant	Concentration (ng/g lipid base) ²	Number of cases (N = 90)	Number of controls (N = 151)	Diminished Ovarian Response Multivariate ¹ logistic regression	
				OR	CI
Organochlorine pesticides					
p,p'-DDE	≤ 22.39	18	51	Ref.	Ref.
]22.39-40.74]	37	50	1.61	0.75 - 3.48
	> 40.74	35	50	1.78	0.80 - 3.97
	Continuous	90	151	1.45	1.11 - 1.90
p,p'-DDT	< LOD	30	51	Ref.	Ref.
	≥ LOD	60	100	0.89	0.47 - 1.68
cis-Heptachlore epoxyde	≤ 1.71	35	50	Ref.	Ref.
]1.71-2.42]	29	51	0.73	0.37 - 1.45
	> 2.42	26	50	0.71	0.35 - 1.41
	Continuous	90	151	0.93	0.56 - 1.54
HCB	≤ 6.43	25	51	Ref.	Ref.
]6.43-8.42]	29	50	0.90	0.44 - 1.87
	> 8.42	36	50	1.09	0.51 - 2.34
	Continuous	90	151	1.03	0.54 - 1.95
β-HCH	≤ 3.62	33	51	Ref.	Ref.
]3.62-5.77]	35	50	0.76	0.38 - 1.51
	> 5.77	22	50	0.38	0.17 - 0.82
	Continuous	90	151	0.50	0.32 - 0.76
Polychlorinated biphenyls					
PCB 28	≤ 0.41	25	49	Ref.	Ref.
]0.41-0.60]	29	51	1.07	0.39 - 2.92
	> 0.60	36	51	1.41	0.63 - 3.14
	Continuous	90	151	1.03	0.70 - 1.50
PCB 74	≤ 1.65	22	51	Ref.	Ref.
]1.65-2.52]	33	49	1.19	0.57 - 2.48
	> 2.52	35	51	1.12	0.52 - 2.41
	Continuous	90	151	0.90	0.63 - 1.29
PCB 99	≤ 1.82	22	51	Ref.	Ref.
]1.82-2.69]	30	50	1.44	0.68 - 3.01
	> 2.69	38	50	1.65	0.78 - 3.50
	Continuous	90	151	1.05	0.69 - 1.59
PCB 118	≤ 3.45	18	51	Ref.	Ref.
]3.45-5.50]	38	50	1.84	0.88 - 3.86
	> 5.50	34	50	1.51	0.70 - 3.28
	Continuous	90	151	0.96	0.63 - 1.46
PCB 138+163 ³	≤ 3.71	24	51	Ref.	Ref.
]3.71-6.44]	23	50	0.81	0.37 - 1.78
	> 6.44	43	50	1.30	0.54 - 3.13

PCB 153	Continuous	90	151	1.03	0.67 - 1.58
	≤ 16.36	22	51	Ref.	Ref.
]16.36-26.21]	32	50	1.22	0.57 - 2.62
	> 26.21	36	50	1.15	0.49 - 2.67
PCB 170	Continuous	90	151	1.08	0.67 - 1.74
	≤ 11.34	21	51	Ref.	Ref.
]11.34-16.43]	26	50	1.03	0.48 - 2.21
	> 16.43	43	50	1.65	0.74 - 3.68
PCB 180	Continuous	90	151	1.05	0.67 - 1.66
	≤ 10.85	21	51	Ref.	Ref.
]10.85-18.91]	28	50	1.04	0.47 - 2.30
	> 18.91	41	50	1.28	0.51 - 3.19
PCB 183	Continuous	90	151	1.10	0.71 - 1.70
	≤ 0.94	20	51	Ref.	Ref.
]0.94-1.52]	33	51	1.46	0.68 - 3.11
	> 1.52	37	49	1.45	0.63 - 3.33
PCB 187	Continuous	90	151	1.21	0.77 - 1.89
	≤ 2.06	27	52	Ref.	Ref.
]2.06-3.54]	29	52	1.06	0.48 - 2.35
	> 3.54	34	47	1.38	0.61 - 3.09
PCB 194	Continuous	90	151	1.11	0.78 - 1.58
	≤ 1.23	21	52	Ref.	Ref.
]1.23-2.38]	35	49	1.20	0.54 - 2.65
	> 2.38	34	50	0.97	0.38 - 2.48
PCB 196+203 ³	Continuous	90	151	1.16	0.76 - 1.77
	≤ 1.12	20	51	Ref.	Ref.
]1.12-1.89]	33	51	1.30	0.58 - 2.92
	> 1.89	37	49	1.39	0.56 - 3.47
	Continuous	90	151	1.22	0.79 - 1.89

¹Adjusted for age, body-mass index, centre, education level, and season of inclusion.

²Log2-transformed. Continuous analyses were only performed when linearity was respected.

³These POPS could not be distinguished during the measurement process.

Abbreviations: OR, odds ratio; CI, confidence interval; Ref, reference.

Supplementary Table SVII. Sensitivity analysis – Associations between serum persistent organic pollutants and diminished ovarian reserve in the AROPE study – No prior stimulation treatment (N = 97 cases & 97 controls)

Persistent organic pollutant	Concentration (ng/g lipid base) ²	Number of cases (N = 97)	Number of controls (N = 97)	Diminished Ovarian Reserve Multivariate ¹ logistic regression	
				OR	CI
Organochlorine pesticides					
p,p'-DDE	≤ 21.98	22	33	Ref.	Ref.
]21.98-37.32]	33	32	1.25	0.56 - 2.80
	> 37.32	42	32	1.71	0.76 - 3.83
	Continuous	97	97	1.55	1.14 - 2.11
p,p'-DDT	< LOD	37	36	Ref.	Ref.
	≥ LOD	60	61	1.20	0.62 - 2.33
cis-Heptachlore epoxyde	≤ 1.75	39	33	Ref.	Ref.
]1.75-2.37]	31	31	0.65	0.30 - 1.39
	> 2.37	27	33	0.59	0.28 - 1.26
	Continuous	97	97	0.66	0.37 - 1.16
HCB	≤ 6.69	31	33	Ref.	Ref.
]6.69-8.70]	33	32	0.80	0.37 - 1.74
	> 8.70	33	32	0.75	0.33 - 1.68
	Continuous	97	97	0.85	0.41 - 1.73
β-HCH	≤ 3.52	31	33	Ref.	Ref.
]3.52-5.66]	38	32	0.76	0.35 - 1.64
	> 5.66	28	32	0.45	0.19 - 1.06
	Continuous	97	97	0.68	0.44 - 1.03
Polychlorinated biphenyls					
PCB 28	≤ 0.42	29	36	Ref.	Ref.
]0.42-0.59]	28	28	0.96	0.39 - 2.33
	> 0.59	40	33	1.40	0.63 - 3.11
	Continuous	97	97	1.27	0.80 - 2.03
PCB 74	≤ 1.61	25	33	Ref.	Ref.
]1.61-2.52]	32	31	0.98	0.43 - 2.24
	> 2.52	40	33	0.96	0.42 - 2.21
	Continuous	97	97	0.88	0.58 - 1.35
PCB 99	≤ 1.81	28	33	Ref.	Ref.
]1.81-2.66]	27	31	0.92	0.40 - 2.08
	> 2.66	42	33	1.30	0.58 - 2.93
	Continuous	97	97	0.99	0.62 - 1.59
PCB 118	≤ 3.31	18	33	Ref.	Ref.
]3.31-5.32]	43	32	1.91	0.87 - 4.22
	> 5.32	36	32	1.37	0.59 - 3.17
	Continuous	97	97	0.91	0.58 - 1.44
PCB 138+163 ³	≤ 3.67	22	33	Ref.	Ref.
]3.67-6.30]	29	32	0.94	0.40 - 2.24
	> 6.30	46	32	1.16	0.42 - 3.20

PCB 153	Continuous	97	97	1.00	0.61 - 1.64
	≤ 15.87	23	33	Ref.	Ref.
]15.87-26.21]	35	32	1.00	0.43 - 2.34
	> 26.21	39	32	1.01	0.39 - 2.57
PCB 170	Continuous	97	97	1.01	0.59 - 1.72
	≤ 11.06	24	33	Ref.	Ref.
]11.06-16.43]	22	32	0.70	0.30 - 1.63
	> 16.43	51	32	1.60	0.67 - 3.80
PCB 180	Continuous	97	97	1.00	0.60 - 1.67
	≤ 10.91	20	33	Ref.	Ref.
]10.91-18.13]	28	32	1.04	0.43 - 2.56
	> 18.13	49	32	1.35	0.47 - 3.84
PCB 183	Continuous	97	97	1.07	0.65 - 1.78
	≤ 0.93	23	33	Ref.	Ref.
]0.93-1.39]	20	32	0.69	0.29 - 1.67
	> 1.39	54	32	1.91	0.77 - 4.71
PCB 187	Continuous	97	97	1.36	0.82 - 2.27
	≤ 2.05	27	35	Ref.	Ref.
]2.05-3.50]	29	32	0.99	0.42 - 2.31
	> 3.50	41	30	1.31	0.53 - 3.20
PCB 194	Continuous	97	97	1.14	0.77 - 1.71
	≤ 1.20	21	32	Ref.	Ref.
]1.20-2.22]	30	32	0.93	0.37 - 2.31
	> 2.22	46	33	1.09	0.37 - 3.20
PCB 196+203 ³	Continuous	97	97	1.15	0.70 - 1.89
	≤ 1.10	19	32	Ref.	Ref.
]1.10-1.78]	35	33	1.32	0.52 - 3.31
	> 1.78	43	32	1.34	0.45 - 4.04
	Continuous	97	97	1.33	0.80 - 2.22

¹Adjusted for age, body-mass index, centre, education level, and season of inclusion.

²Log₂-transformed. Continuous analyses were only performed when linearity was respected.

³These POPS could not be distinguished during the measurement process.

Abbreviations: OR, odds ratio; CI, confidence interval; Ref, reference.

Supplementary Table SVIII. Sensitivity analysis – Associations between serum persistent organic pollutants and diminished ovarian reserve in the AROPE study – Participants with outcomes measured at inclusion (N = 50 cases & 48 controls)

Persistent organic pollutant	Concentration (ng/g lipid base) ²	Number of cases (N=50)	Number of controls (N=48)	Diminished Ovarian Response	
				OR	CI
Organochlorine pesticides					
p,p'-DDE	≤ 21.33	9	16	Ref.	Ref.
]21.33-35.63]	15	16	1.21	0.31 - 4.73
	> 35.63	26	16	2.60	0.72 - 9.43
	Continuous ²	50	48	1.36	0.83 - 2.24
p,p'-DDT	< LOD	17	13	Ref.	Ref.
	≥ LOD	33	35	1.44	0.49 - 4.27
cis-Heptachlore-Epoxyde	≤ 1.75	19	16	Ref.	Ref.
]1.75-2.48]	22	16	0.66	0.22 - 1.98
	> 2.48	9	16	0.51	0.14 - 1.83
	Continuous	50	48	0.98	0.39 - 2.42
HCB	≤ 6.52	14	16	Ref.	Ref.
]6.52-7.99]	12	16	0.61	0.18 - 2.08
	> 7.99	24	16	1.09	0.34 - 3.55
	Continuous	50	48	1.48	0.43 - 5.15
Beta-HCH	≤ 3.64	13	16	Ref.	Ref.
]3.64-6.41]	26	16	1.88	0.59 - 6.01
	> 6.41	11	16	0.60	0.15 - 2.51
Polychlorinated biphenyls					
PCB 28	≤ 0.39	13	16	Ref.	Ref.
]0.39-0.53]	21	16	2.27	0.45 - 11.33
	> 0.53	16	16	1.96	0.39 - 9.94
	Continuous	50	48	1.06	0.55 - 2.04
PCB 74	≤ 1.61	10	16	Ref.	Ref.
]1.61-2.90]	25	17	2.57	0.71 - 9.31
	> 2.90	15	15	1.34	0.34 - 5.28
PCB 99	≤ 1.81	8	16	Ref.	Ref.
]1.81-2.50]	15	16	1.26	0.35 - 4.54
	> 2.50	27	16	2.47	0.73 - 8.33
	Continuous	50	48	1.33	0.65 - 2.72
PCB 118	≤ 3.31	8	16	Ref.	Ref.
]3.31-5.01]	19	16	2.90	0.76 - 11.08
	> 5.01	23	16	3.83	0.99 - 14.87
	Continuous	50	48	1.37	0.69 - 2.72
PCB 138 + 163 ³	≤ 11.68	9	16	Ref.	Ref.
]11.68-15.43]	14	16	1.30	0.35 - 4.83
	> 15.43	27	16	2.33	0.65 - 8.33
	Continuous	50	48	1.53	0.67 - 3.52
PCB 153	≤ 17.15	10	16	Ref.	Ref.

]17.15-24.18]	12	16	0.69	0.18 - 2.64
	> 24.18	28	16	1.80	0.49 - 6.57
PCB 170	≤ 4.23	14	16	Ref.	Ref.
]4.23-6.38]	13	16	0.60	0.16 - 2.28
	> 6.38	23	16	1.20	0.31 - 4.71
	Continuous	50	48	1.26	0.54 - 2.91
PCB 180	≤ 12.81	14	16	Ref.	Ref.
]12.81-18.86]	16	16	0.72	0.20 - 2.67
	> 18.86	20	16	1.00	0.25 - 3.97
	Continuous	50	48	1.13	0.48 - 2.69
PCB 183	≤ 0.94	8	16	Ref.	Ref.
]0.94-1.34]	12	16	0.99	0.25 - 3.96
	> 1.34	30	16	2.55	0.68 - 9.54
	Continuous	50	48	1.82	0.80 - 4.15
PCB 187	≤ 2.07	12	16	Ref.	Ref.
]2.07-3.31]	18	15	1.41	0.37 - 5.40
	> 3.31	20	17	1.75	0.45 - 6.79
	Continuous	50	48	1.55	0.77 - 3.13
PCB 194	≤ 1.40	13	16	Ref.	Ref.
]1.40-2.37]	22	16	1.48	0.43 - 5.14
	> 2.37	15	16	0.81	0.18 - 3.67
PCB 196 + 203 ³	≤ 1.19	13	16	Ref.	Ref.
]1.19-1.88]	20	16	0.74	0.21 - 2.63
	> 1.88	17	16	0.79	0.19 - 3.37

¹Adjusted for age, body-mass index, centre, education level, and season of inclusion.

²All POPs were log₂-transformed when treated as continuous variables.

³These POPs could not be distinguished during the measurement process.

Abbreviations: N, number of subjects; OR, odds ratio; CI, confidence interval; Ref, reference.