Additional effect of drinking water endocrine-disrupting compound exposure on fetal growth compared to the effect of active tobacco exposure during pregnancy

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Abstract

Background

Active tobacco exposure during pregnancy is known to be an important determinant of fetal growth. Nitrates and atrazine metabolites in drinking water may affect fetal growth as a mixture of endocrine disruptors (ED). Our aim was to determine whether ED have an additional effect on fetal growth compared to the known and observed effect of active tobacco exposure during pregnancy.

Methods

A historic cohort study was carried out with a sample stratified with regard to maternity ward, drinking water exposure and year of birth. The women included were living in Deux-Sèvres, had given birth between 2005 and 2010 in three selected maternity wards, and ultrasound data from pregnancy were available in their obstetrical records. Mixed linear models were used to analyze fetal weight evolution from second trimester of pregnancy to time of birth according to drinking water exposure to atrazine metabolite and nitrate mixture and active tobacco exposure.

Results

We included 558 mother-neonate couples, of whom 8.5% were exposed to high doses of mixture and 20.5% to active tobacco smoking. There was no significant difference in fetal weight evolution according to drinking water mixture exposure (0.97 g; 95% CI [-3.01;4.94]) and when comparing nested models with and without drinking water mixture exposure, the likelihood ratio (LR) test was not significant (LR khi²=6.6, p=0.252).

Conclusion

We could not show a supplementary effect of mixture exposure on fetal growth as compared to active tobacco exposure. Further research is needed, using more precise methods to estimate ED exposure.

Introduction

Adverse pregnancy outcomes such as low birth weight (LBW) and small-for-gestational age (SGA) are known to have effects on neonatal and future adult health. More specifically, these outcomes may increase the risk of cardiovascular disease, type 2 diabetes and psychomotor and cognitive impairment (1). In addition, they may lead to increased perinatal mortality (2).

Several factors are known to have an effect on fetal growth (3): genetic factors such as the infant's gender and ethnic group; constitutional factors such as parents' height and weight; socio-demographic factors including maternal age and parental income; obstetric factors involving parity, infertility, low birth weight in siblings, nutritional intake during pregnancy.

It is a known fact that active tobacco exposure is the only factor responsible for an etiologic fraction of perinatal outcomes, causing 24–25% of intrauterine growth retardation (IUGR) or SGA and 16% of LBW in

developed countries (4,5). Moreover, the effect of tobacco exposure can be observed as soon as the second trimester of pregnancy on head circumference and femur length (6). Several hypotheses have been put forward on the effect of active tobacco exposure on fetal growth: 1: direct toxicity on fetal growth; 2: nutritional deprivation due to slowed blood circulation; 3: context of other risk factors for adverse pregnancy outcomes associated with maternal smoking during pregnancy (7,8).

While the second hypothesis is predominantly favored in the literature, the third hypothesis is more and more relevant. Tobacco smoke contains an endocrine disruptor mixture that could have antagonist or synergistic effects. Tobacco has an anti-estrogenic effect on women by inhibiting aromatase activity and by estradiol metabolite hydroxylation (9). Lower levels of estradiol are measured in newborns exposed to active tobacco during pregnancy. In addition, three pesticides of the dinitroaniline family have been detected in tobacco smoke (10). These three pesticides are known or suspected endocrine disruptors.

Other environmental air or water pollutants are suspected to have an effect on fetal growth during pregnancy. Outdoor air pollution and retarded fetal growth have been associated in the literature: SGA prevalence was higher in women exposed to high doses of particulate with aerodynamic diameter < 10 µm (PM_{10}) during pregnancy (11) and fetal growth between 20 and 32 weeks was lower among mothers exposed to nitrogen dioxide (NO_2) during pregnancy (12), with a critical window of exposure in early pregnancy, prior to 20 weeks of gestation. Moreover, CO and NO_2 prenatal exposure was associated with an increased risk of SGA, particularly for exposures early in pregnancy, during the first month of gestation and the first trimester (13).

Drinking water exposure to environmental pollutants has also been studied, and associations between drinking water exposure to endocrine disruptors and SGA or LBW have been discovered: mothers exposed during pregnancy to atrazine (14–18) or a mixture of atrazine metabolites and nitrates (19) in drinking water were more likely to give birth to SGA or LBW babies.

Nitrates are potential EDCs with an anti-androgenic effect (20,21) by inhibiting the steroid hormone syntheses via conversion to nitric oxide. Nitric oxide inhibits the cytochrome P450 enzymes stopping the transformation of free cholesterol into progesterone (22). Atrazine is likewise a potential EDC with an anti-androgenic and a weak estrogenic effects (23). There may therefore exist an additive effect of active tobacco exposure when mixed with environmental exposure to EDC during pregnancy. Indeed, EDCs have specific properties such as synergism or antagonism when mixed, which depend on the dose (24) and on each substance's mode of action (25). In the literature, most studies have highlighted the effect on fetal growth of a single compound (tobacco, pesticides), while two studies have focused on a mixture exposure of atrazine metabolite and nitrate (19,26).

A dose-response relationship between active tobacco exposure and fetal growth has been repeatedly observed (5,27,28), and involves causality. The effect of maternal tobacco smoking on fetal growth has been described as early as 18 weeks of amenorrhea (WA) on ultrasound biometry measurements and could vary according to the time period of pregnancy, thereby introducing the notion of window exposure effect on fetal growth (28,29). Definitions of outcomes in the literature have nonetheless differed (low birth weight, below 2500 g, SGA and IUGR) as have the exposure periods studied (entire pregnancy, second trimester, third

trimester). All of them have used fetal growth measurements at birth, particularly birth weight, the usual indicator of fetal growth. Because of these discrepancies, no period of greater vulnerability of the fetus or exposure window during pregnancy has been found. However, fetal growth can be assessed early in pregnancy with ultrasound measurements: biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL). These biometry parameters can be used to calculate estimated fetal weight (EFW), according to mathematical formulas (30). Biometry parameters enable searching for exposure effects during pregnancy, before birth and at specific times, according to the exposures of interest.

Our aim was to determine whether exposure to endocrine-disrupting compounds in drinking water has an additional effect on fetal growth measured by fetal weight compared to the known and observed effect of active tobacco exposure during pregnancy.

Methods

This retrospective cohort study was conducted between 2005 and 2010 in Deux-Sèvres, in western France. In this district of 5999 km² with 362944 inhabitants in 2007, agricultural activity is predominant with 75% of land use essentially involving livestock, mainly sheep and goats along with cereal production. We chose Deux-Sèvres because of its rurality and consequently sizable use of pesticides and also because of the highest known ground concentration of nitrates in the western part of France.

The French regional health agency (ARS, Agence Régionale de Santé) regularly assesses pesticide and nitrate levels in drinking water. As required by law, the number of measurements by municipality is proportional to population size. Municipalities are split or grouped into community water systems (CWS), geographic areas receiving drinking water from the same source, and water quality is considered to be homogeneous within a CWS. All the samples concerned treated water, and were taken from CWS and water treatment plants, between 1 April 2004 and 31 December 2010 in order to obtain complete exposure data throughout the pregnancy of every woman included in the study.

Birth records are drawn from the infant health certificates issued by the district office of maternal and childhood protection. Completed by the hospital or clinic before the infant's discharge, these documents are mandatory, and thereby likely to include practically all births. The information contained in these certificates includes sex, birth weight, gestational age (weeks of amenorrhea at birth), age of mother, number of previous pregnancies, parental occupation and place of residence at birth. Data from birth records were validated according to a methodology approved by the French "Direction de la recherche, des études, de l'évaluation et des statistiques" (31).

We identified all live births in Deux-Sèvres from 1 January 2005 to 31 December 2010 of neonates whose mothers lived in the district at the time of birth, whose birth took place in the maternity wards of the hospitals of Niort, Poitiers and Bressuire and whose birth certificate had been recorded. We excluded the non-environmental causes known to induce low birth weight such as multiple births, early deaths (before birth record completion), neonates with congenital abnormalities and birth by cesarean section. We also excluded neonates whose mothers lived in municipalities having more than one CWS providing drinking water or whose place of residence could not be identified. Sampling date, sampling location and CWS or treatment

plant names were available for each measurement in drinking water. Individual nitrate and pesticide exposure levels were assigned by the maternal municipality of residence at birth. The exposure variable and the individual data were merged by the place of residence of the mother at birth.

Because information about pregnancy ultrasounds and certain maternal characteristics are not available in birth records, we went to maternity wards to collect supplementary data in the obstetrical records. For feasibility reasons, we selected a random sample of mother-neonate couples, stratified with regard to year of birth, exposure status to atrazine metabolites during pregnancy and maternity ward of birth.

The number of subjects needed to show a difference of 100 grams of fetal weight at third trimester between couples exposed and couples not exposed to atrazine metabolites with a power of 80% was 800 mother-neonate couples. The choice of the difference was based on two of the quantified effects of environmental exposure on EFW at third trimester found in the literature: active tobacco exposure with a difference in EFW of 56 g (7) and outdoor air pollution exposure with a difference in EFW of 200 g (12). In the absence of other data in the literature, we chose between the values of tobacco and air pollution, at 100 g.

Data about ultrasounds, gestational diabetes, smoking during pregnancy and maternal weight and height were obtained in the obstetrical records of the mothers sampled. At this step we excluded mother-neonate couples whose files did not contain detailed ultrasound reports from second and third trimester and cases in which ultrasound measurements were carried out before 20 or after 25 weeks of amenorrhea (WA) for second trimester and before 30 and after 35 WA for third trimester. We also excluded births occurring before 35 WA so that there was no overlap of gestational age between estimated fetal weight at third trimester and birth weight.

Our principal outcome consisted in the evolution of fetal weight between second trimester and birth. Estimated fetal weight at second and third trimesters of pregnancy was calculated by a mathematical formula based on fetal biometry parameters: biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL), given by the results of pregnancy ultrasounds. This formula was developed by Hadlock (32): Log10(PFE)= 1.335+0,0316.BPD+ 0.0457.AC+0.1623.FL-0.0034.AC.FL. Birth weight was collected in birth records.

Two types of exposure during pregnancy were studied: drinking water exposure to a mixture of atrazine metabolites (pesticides most often found in drinking water) and nitrates defined according to a method described elsewhere (19) and active tobacco smoking of the mother during pregnancy.

Mixture exposure was defined by six classes (unexposed to pesticides and exposed to first tercile of mean concentration of nitrates; unexposed to pesticides and exposed to second tercile of mean concentration of nitrates; unexposed to pesticides and exposed to third tercile of mean concentration of nitrates; exposed to pesticides and to first tercile of mean concentration of nitrates; exposed to pesticides and to second tercile of mean concentration of nitrates; exposed to pesticides and to third tercile of mean concentration of nitrates).

Active tobacco exposure during pregnancy was defined as a binary variable: yes/no.

Univariate and bivariate analyses were conducted incorporating sample probabilities according to the stratification used and calculated weight differences in grams and 95% confidence intervals (95%CI).

Covariates considered in multivariate analysis were known or suspected factors acting on birth weight or factors influencing drinking water exposure to pesticides and nitrates. They included covariates acting on birth weight : newborn gender; maternal weight before pregnancy (in kilograms); maternal age (<27; 27-29; 29-33; >33 years); history of low birth weight in siblings (yes/no); household occupation (disadvantaged: workers and unemployed; moderately advantaged: self-employed, employees and farmers; advantaged: managers and executives); gestational diabetes; gestational age at ultrasound examination or birth (weeks of amenorrhea) and factors influencing drinking water exposure: rural location of residence at birth; season during which second trimester of pregnancy took place. We included rural and seasonal variables in the analyses because pesticide usage is predominant in summer and autumn and in rural areas.

Fetal weight evolution between second trimester and birth was studied by longitudinal data analysis using three linear mixed models: one model with drinking water exposure to the mixture of atrazine metabolites and nitrates, another model with active tobacco exposure and a third model with both exposures. The outcome was modeled as the exponential of gestational age in weeks. Random effects on intercept and slope (weeks of gestational age) were allowed. Goodness of fit was assessed by consideration of independence and normality of the residuals (33).

We compared nested models using the likelihood ratio (LR) test (34) and non-nested models using the Akaike criteria (AIC).

Analyses were conducted with SAS (version 9.3; SAS Institute, Cary, NC, USA®).

Results

Among the study population of 14,022 mother-neonate couples, 800 were randomly sampled and 558 were finally included in the study, as shown in the flowchart (Fig. 1).

Among the 558 mother-neonate couples, the average number of atrazine metabolite measurements in drinking water during pregnancy was 3 ± 1 and the average number of nitrate measurements was 35 ± 30.

During the study period, 438 drinking water atrazine metabolite measurements were carried out; for desethylatrazine and 2hydroxyatrazine (the most frequent metabolites) they ranged from 0 to 0.1 µg/L. Most of them (432, 98.4%) came from treatment plant water. During pregnancy, 283 mothers (corresponding to 50.7% of the raw total and to 42.5% of the weighted total) were exposed to atrazine metabolites with an average concentration of atrazine metabolites in drinking water of 0.04 ± 0.02 µg/L for both desethylatrazine and 2hydroxyatrazine.

During the study period, 3168 drinking water nitrate measurements were performed; they ranged from 0 to 63.3 mg/L and came from CWS waters only. During pregnancy, all mothers were exposed to nitrates with an

average concentration of nitrates in drinking water of 23.5 ± 11.8 mg/L. Limits of drinking water nitrate concentration terciles were 18.1 and 30.3 mg/L during the entire pregnancy.

Study population characteristics are presented in Table 1.

Table 1

Characteristics of the study population and potential confounders, Deux-Sèvres, France.

	Raw results				**Weighted results***	
	Missing values		Total (N = 558)		Total (N = 9013)	
	N	%	N	%	N	%
Sex of the newborn	0	0				
Boy			238	42.7	3893	43.2
Girl			320	57.4	5120	56.8
Premature birth	0	0				
Yes			16	2.9	282	3.1
Household occupation	5	1				
Disadvantaged			69	12.5	1157	13.0
Moderatly advantaged			352	63.7	5932	66.5
Advantaged			132	23.9	1825	20.5
Rural location of residence	0	0				
Yes			206	36.9	2974	33.0
Primiparity	13	2				
Yes			215	39.5	3329	37.6
Maternal age (years)	0	0				
< 27			135	24.2	2211	24.5
27–29			124	22.2	2144	23.8
30–33			154	27.6	2497	27.7
> 33			145	26.0	2161	24.0
Maternal body mass index (kg/m²)	6	1				
< 18			24	4.4	395	4.5
18–24			376	68.1	5858	66.1
25–29			91	16.5	1605	18.1
> 29			61	11.1	1011	11.4
History of low birthweight	7	1				

	Raw results				Weighted results*	
Yes			20	3.6	314	3.5
Gestational diabetes	49	9				
Yes			42	8.3	685	8.3
Season during second trimester	0	0				
Spring			124	22.2	2131	23.6
Summer			146	26.2	2214	24.6
Fall			132	23.7	2193	24.3
Winter			156	28.0	2475	27.5
Smoking during pregnancy	50	9				
Yes			110	21.7	1665	20.5
Mixture exposure during whole pregnancy	0	0				
Atrazine metabolites No and Nitrates < 18.14 mg/L			66	11.8	1224	13.6
Atrazine metabolites No and Nitrates 18.14–30.33 mg/L			137	24.6	2679	29.7
Atrazine metabolites No and Nitrates > 30.33 mg/L			72	12.9	1279	14.2
Atrazine metabolites Yes and Nitrates < 18.14 mg/L			120	21.5	2532	28.1
Atrazine metabolites Yes and Nitrates 18.14–30.33 mg/L			54	9.7	538	6.0
Atrazine metabolites Yes and Nitrates > 30.33 mg/L			109	19.5	761	8.5
*Weigth defined by the inverse of the sample probabilities						

Mean estimated fetal weight was 558.2 ± 4.8 grams at second trimester and 2068.6 ± 14.0 grams at third trimester. Mean gestational age at completion of ultrasound measurement was 22.3 ± 0.04 weeks of amenorrhea (WA) at second trimester and 32.3 ± 0.04 WA at third trimester. Mean birth weight was 3337.3 ± 23.5 grams and mean gestational age at birth was 39.4 ± 0.1 WA.

Before adjustment on the available confounders, fetal weight evolution between second trimester and birth was associated with active tobacco exposure during pregnancy but not with drinking water exposure to an atrazine metabolite and nitrate mixture (Table 2).

Table 2

Fetal weight evolution during pregnancy according to drinking water mixture exposure and active tobacco exposure before and after adjustment for available confounders, Deux-Sèvres, France.

	Fetal weight between second trimester and birth (grams)					
	Unadjusted analysis			Adjusted analysis (N = 458)		
	Difference	95%CI	p	Difference	95%CI	p
Weight evolution according to drinking-water mixture exposure during pregnancy			0.512			0.481
Atrazine metabolites No and Nitrates < 18.14 mg/L	1			1		
Atrazine metabolites No and Nitrates 18.14–30.33 mg/L	-0.60	[-4.48;3.28]		-0.76	[-4.56;3.04]	
Atrazine metabolites No and Nitrates > 30.33 mg/L	-1.48	[-6.03;3.06]		-1.60	[-6.06;2.86]	
Atrazine metabolites Yes and Nitrates < 18.14 mg/L	1.98	[-2.03;5.99]		1.85	[-2.08;5.79]	
Atrazine metabolites Yes and Nitrates 18.14–30.33 mg/L	0.02	[-4.62;4.66]		-0.17	[-4.72;4.38]	
Atrazine metabolites Yes and Nitrates > 30.33 mg/L	1.06	[-2.99;5.11]		0.97	[-3.01;4.94]	
Weight evolution according to smoking during pregnancy			0.010			0.009
Yes	-3.48	[-6.13;-0.83]		-3.46	[-6.07;-0.85]	
No	1			1		
Season during second trimester			0.812			0.759

Fetal weight between second trimester and birth (grams)						
Fall	-29.66	[-90.53;31.21]		-31.37	[-91.21;28.48]	
Winter	-15.13	[-73.63;43.37]		-20.92	[-79.78;37.94]	
Spring	-19.46	[-82.84;43.91]		-10.22	[-72.78;52.34]	
Summer	1			1		
Newborn gender			0.004			0.005
Girl	-64.62	[-108.46;-20.78]		-62.26	[-105.98;-18.55]	
Boy	1			1		
Maternal weight before pregnancy (kg)	2.65	[1.09;4.22]	0.001	2.48	[0.91;4.05]	0.002
Maternal age (years)			0.614			0.901
< 27	-21.79	[-85.43;41.85]		7.79	[-58.33;73.91]	
27–29	1			1		
29–33	4.42	[-57.82;66.66]		16.44	[-44.65;77.53]	
> 33	19.89	[-43.38;83.16]		23.01	[-40.84;86.86]	
History of low birth weight			0.579			0.483
Yes	-34.01	[-154.30;86.28]		-43.64	[-165.54;78.26]	
No	1			1		
Household occupation			0.842			0.987
Disadvantageous	-18.74	[-97.09;59.61]		-5.94	[-86.70;74.83]	
Moderately advantageous	2.04	[-50.36;54.45]		-3.68	[-55.72;48.35]	
Advantageous	1			1		
Gestational diabetes			0.108			0.169
Yes	63.25	[-13.97;140.47]		53.76	[-22.95;130.46]	
No	1			1		
Rural location of residence at birth			0.911			0.722
Yes	-2.57	[-47.72;42.58]		-8.69	[-56.66;39.28]	

Fetal weight between second trimester and birth (grams)		
No	1	1
95%CI: 95% confidence interval.		

After adjustment on the available confounders, fetal weight evolution between second trimester and birth was not associated with drinking water exposure to an atrazine metabolites and nitrates mixture. However, fetal weight evolution was associated with tobacco exposure with (-3.46 g per week of amenorrhea [-6.07;-0.85]) or without drinking water exposure to an atrazine metabolites and nitrates mixture (-3.43 g per week of amenorrhea [-6.05;-0.82]) (Table 2 and Table 3). Comparing both models, the likelihood ratio test was not significant (LR khi² test = 6.6, p = 0.25).

Table 3

Comparison of non-nested models of fetal weight evolution according to active tobacco exposure and drinking water mixture exposure during pregnancy, Deux-Sèvres, France.

	Fetal weight between second trimester and birth in grams (N = 458)					
	Only drinking-water exposure AIC = 19658.4			Only tobacco exposure AIC = 19636.7		
	Difference	95%CI	p	Difference	95%CI	p
Weight evolution according to mixture exposure during pregnancy			0.506			
Atrazine metabolites No and Nitrates < 18.14 mg/L	1					
Atrazine metabolites No and Nitrates 18.14–30.33 mg/L	-0.64	[-4.46;3.19]				
Atrazine metabolites No and Nitrates > 30.33 mg/L	-1.52	[-6.00;2.97]				
Atrazine metabolites Yes and Nitrates < 18.14 mg/L	1.93	[-2.02;5.89]				
Atrazine metabolites Yes and Nitrates 18.14–30.33 mg/L	-0.03	[-4.61;4.55]				
Atrazine metabolites Yes and Nitrates >30.33 mg/L	0.98	[-3.01;4.98]				
Weight evolution according to smoking during pregnancy						0.010
Yes				-3.43	[-6.05;-0.82]	
No				1		
Season during second trimester			0.779			0.776

	Fetal weight between second trimester and birth in grams (N = 458)					
Fall	-31.44	[-91.73;28.85]		-29.55	[-89.25;30.15]	
Winter	-19.72	[-79.02;39.58]		-21.55	[-79.65;36.54]	
Spring	-13.96	[-76.89;48.97]		-9.55	[-72.14;53.04]	
Summer	1			1		
Newborn gender			0.010			0.003
Girl	-57.97	[-101.90;-14.05]		-65.23	[-108.87;-21.58]	
Boy	1			1		
Maternal weight before pregnancy (kg)	2.51	[0.93;4.09]	0.002	2.44	[0.87;4.01]	0.002
Maternal age (years)			0.749			0.895
< 27	-8.98	[-74.13;56.17]		7.24	[-58.83;73.30]	
27–29	1			1		
29–33	12.51	[-48.96;73.98]		16.12	[-45.03;77.27]	
> 33	24.40	[-39.93;88.73]		23.29	[-40.06;86.64]	
History of low birth weight			0.340			0.450
Yes	-59.38	[-181.49;62.74]		-46.74	[-167.99;74.51]	
No	1			1		
Household occupation			0.862			0.998
Disadvantageous	-22.24	[-102.38;57.90]		-2.29	[-82.81;78.23]	
Moderately advantageous	-6.58	[-58.93;45.77]		-0.58	[-52.38;51.23]	
Advantageous	1			1		
Gestational diabetes			0.217			0.156
Yes	48.63	[-28.54;125.80]		55.25	[-21.10;131.60]	
No	1			1		
Rural location of residence at birth			0.791			0.692
Yes	-6.51	[-54.81;41.79]		-9.04	[-53.90;35.82]	

	Fetal weight between second trimester and birth in grams (N = 458)	
No	1	1
95%CI: 95% confidence interval; AIC: Akaike criterion.		

Discussion

Our results did not show that drinking water exposure to an EDC mixture has a supplementary adverse effect on fetal growth between second trimester and birth when active tobacco exposure is likewise taken into consideration.

We found a decrease in fetal weight evolution between second trimester of pregnancy and birth when the mother smoked during pregnancy (-3.46 g per week of amenorrhea [-6.07;-0.85]). The decrease in fetal weight evolution during pregnancy of 3.46 grams per week of amenorrhea (WA) represents a difference of 138 grams at birth (40 WA) between exposed and unexposed neonates. This result is consistent with the literature. Jaddoe and al (28) observed a difference of 200 g at birth between neonates actively exposed and neonates not exposed to tobacco during pregnancy, and Gaillard and al noted a difference of 165 g at birth (40 SA) (35). More recently, Cardenas et al (36) observed a difference of 175 g at birth between exposed and unexposed neonates. Furthermore, they observed that prenatal maternal smoking might interact with placental DNA methylation at specific loci in the epigenome, mediating the association with lower birth weight in infants.

We did not find a significant association between fetal weight evolution and drinking water exposure to atrazine metabolites and nitrate mixture. Almberg et al (18) observed an increased risk of low birth weight associated with atrazine exposure in drinking water over the entire gestational period OR 1.27 1.10–1.45) and the first (OR 1.20 1.08–1.34) and second trimester (OR 1.13 1.07–1.20) of pregnancy. However, no association was observed between SGA and atrazine exposure in drinking-water.

The scarcity of significant results in our study may be due to lack of power. We chose a sample size based on the results of the effect of active tobacco exposure (7,37) and outdoor air pollution exposure (12) on fetal growth during pregnancy even though, to our knowledge, there exists no study quantifying the effect on fetal growth of drinking water exposure to pesticides and nitrates, taken either separately or in a mixture. And since at present there is no widely accepted general standard for sample size computations in mixed linear models presenting both fixed and random effects, it was with the method used for multiple linear models that we determined the sample size.

The study population was drawn from birth records that may be considered exhaustive insofar as they are mandatory in France. Sample selection was stratified on drinking water pesticide exposure status according to the results of a prior study (19), year and maternity ward of birth. Stratification on year and maternity ward of birth was carried out with equal probability. We stratified on year of birth in order to take account of

pesticide level variations over the years, since atrazine and its metabolites are more pronouncedly present in drinking water in the district of Deux–Sèvres and since in Europe atrazine has been forbidden from sale since 2002 and from use since 2003 (38). We stratified on maternity ward of birth for feasibility reasons. Mother-neonate couples exposed to pesticides were over-represented in the sample because exposure prevalence in the population is low. Stratification was taken into account in the analysis by weighting on sample probability and stratifying on birth year according to a method described elsewhere (39). Any selection bias was thereby avoided.

Our study population is representative of the general French population in 2010, particularly with regard to the prevalence of mothers smoking during pregnancy (40).

We studied the effects of a mixture exposure of atrazine metabolites and nitrates, both of them endocrine-disrupting compounds that have specific properties such as dose response (41) and synergistic effects (24) when mixed. We did not study the effect of drinking water exposure to atrazine metabolites or nitrates alone because exposure to only one EDC does not reflect reality and consequently does not seem relevant (19).

Assessment of drinking-water exposure is very limited in birth cohorts. Indeed, water contaminants have received the least attention among all other environmental risks in the literature. However, pregnant and lactating women may consume more drinking water than non-pregnant women, increasing their daily intake of water contaminants (42).

However, our assessment of drinking water exposure may not reflect the actual exposure of pregnant women. Laboratory analyses of water quality data do not require a detection limit because their goal is to verify whether or not concentrations are above the regulatory limit, which is largely above the detection limit. In our study, the data situated between detection and quantification limits were considered as absence of exposure to atrazine metabolites. It is possible that we underestimated exposure to atrazine metabolites and subsequently to a mixture and that conversely, we overestimated the association between mixture exposure and fetal growth.

Furthermore, drinking water exposure depends on drinking water consumption patterns. In the western part of France, where the district of Deux-Sèvres is located, the percentage of people drinking tap water was estimated in 2007 at 61% (43). In the United States, it has been observed that pregnant and non-pregnant women do not differ in tap water consumption (44). Furthermore, recent results from the Endocrine Disruptor Deux-Sèvres (EDDS) cohort study showed that 71% of pregnant women drank tap water (45) so it may not have affected our estimates. Besides, using ecological data on drinking-water prevents selection bias that can be found in cohort studies.

In the birth records placed at our disposal, no data were available on women's mobility during pregnancy. All we had at our disposal was the mother's home address at the time of birth. However, moving during pregnancy occurs in only 9–32% of cases, mostly during the second trimester and for a short distance (< 10 km) (46,47) so we can assume that it did not significantly affect our exposure estimates.

Our exposure assessment was not an individual estimation of exposure, which nonetheless seems to be the best method. EDC exposure assessment can be rendered more precise through exposure biomarkers such as blood and urine or cord blood, amniotic fluid and breast milk, with concentrations that could reflect fetal exposure (17,48). For example, BPA and its chlorinated derivatives have been successfully detected and reliably quantified in human urine and colostrum, and the methods applied can be used to estimate fetal exposure to these EDC in drinking water (49,50). Moreover, BPA and phthalate urinary levels have been combined with ultrasound measurements of fetal growth in the literature (51). Atrazine is particularly lipophilic and accumulates in mammary tissues in rats, according to the amount of dose administered and transferred to the offspring via milk (52). However, temporal and spatial variability and the limited samples of the individual estimation method (48) show that ecological assessment, although less accurate, is still useful. Moreover, urinary atrazine metabolites were significantly correlated to tap water consumption during pregnancy (53).

Data on mother-neonate couples were limited to the information available on birth and obstetrical records. Smoking status during pregnancy was binary (yes/no). We did not have enough information in obstetrical records on the number of cigarettes smoked so we could not evaluate the dose of active tobacco exposure. But most other studies have likewise compared smoking to non-smoking mothers and focused on the exposure window (7,29,37). Another limitation of our study consists in the fact that data on active smoking during pregnancy was declarative, which may have induced misclassification of exposure and an under or overestimation of the association. The ultrasound measurements that we used to build our outcome allow for study of fetal growth early in pregnancy, well before birth and therefore for an earlier detection of issues. Other studies assessing the association between drinking water pesticide or nitrate exposure and fetal growth have applied indicators of the latter at the time of birth (15,16,18,19,54), not earlier in pregnancy.

We could not use a formula with all biometry parameters because of the unavailability in obstetrical records of ultrasound measurements of head circumference. We chose Hadlock's formula with three parameters of biometry to determine EFW (30). Even though the equation was obtained from data based on a small number of subjects and although estimation becomes less precise with distance from term, this formula has the same mean absolute error as the formula involving all parameters (55). If we did not have information on the ultrasound operators, the measurement methods or the machines used, it was because this data was not available in the obstetrical records. The measurement variability derived from this lack of information could decrease the precision of our estimations. But the literature shows a good inter and intra-observer reproducibility (56,57), so the lack of precision in our estimations of fetal weight should be limited.

Estimation of fetal weight at any gestational age would be more precise with magnetic resonance imaging and volumetric equations (56) but access to this highly specific technique is distinctly limited in current practice. Nevertheless, the formula used in this study is among the most precise ones available and also one of the most widely used in clinical practice (55,58).

A major strength of our study consists in its repeated measurements of fetal biometry, which facilitate observation of effects at different stages of pregnancy and consequently allow for detailed study of fetal development over time using longitudinal models and taking inter-individual variability and intra-individual

correlation into close consideration. To our knowledge, there exist only a few studies using a similar approach (7,59–62) but none of them have analyzed the effects of presence or absence of active tobacco exposure along with drinking water exposure to an EDC mixture. Unfortunately, such a method implies that exposure effect on fetal growth is homogeneous during pregnancy and the result is averaged, without considering potential time exposure windows (63) when the fetus could be more vulnerable and likewise without considering fetal growth physiology (64) with a greater weight gain at third trimester. It may induce misclassification of exposure and underestimation of the association when an actual window of exposure exists.

Atrazine exposure was measured only in drinking water, which meant that airborne or food exposure could not be assessed. However, it has been shown that atrazine metabolites are rarely found in food and that their atmospheric concentration is usually quite low, just above the detection limit (65). We did not take into account environmental tobacco exposure, but this factor does not modify the association between active tobacco exposure and prevalence of small for gestational age or low birth weight newborns (66) nor is it associated with fetal growth earlier in pregnancy (7). We lacked data on maternal alcohol consumption and nutritional behavior, factors which can also have an effect on fetal growth (3). But the available data was of attested good quality, having been subjected to quality control with validated procedure.

A previous study with an ecological estimation found an association between EDC mixture exposure during pregnancy and birth weight (19). Although it was not observed earlier in pregnancy, a possible effect of EDC mixture on fetal growth may exist, and it should help to motivate preventive actions towards pregnant women on EDC (67).

Conclusion

This historical cohort study did not show that drinking water exposure to an EDC mixture may have an additional adverse effect on fetal growth between second trimester and birth in the event of active tobacco exposure, even though previous works had found that an EDC mixture may affect birth weight. Future studies are needed, with larger samples and using endocrine-disrupting compound biomarkers in blood and breast milk associated with a more individualized assessment of drinking-water exposure with the aim of providing more precise and unbiased fetal exposure estimates. Furthermore, in order to better understand fetal growth dynamics, more complex methods such as prenatal growth curves could constitute valuable modeling, taking into account an appreciable number of fetal and parental characteristics.

Declarations

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CONFLICT OF INTEREST

The authors declare they have no competing interests.

PATIENT CONSENT

Obtained

ETHICS APPROVAL

Comité consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé (CCTIRS); Commission Nationale de l'Informatique et des Libertés (CNIL).

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIAL

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

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No funding was granted for this study

AUTHOR'S CONTRIBUTION

CC elaborated the study design, collected and analyzed the data, wrote the manuscript

ML was a major contributor in the development of the study design, the recruitment of patients and the data collection, the data analysis.

VM supervised all the work

All authors read and approved the final manuscript

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Figures

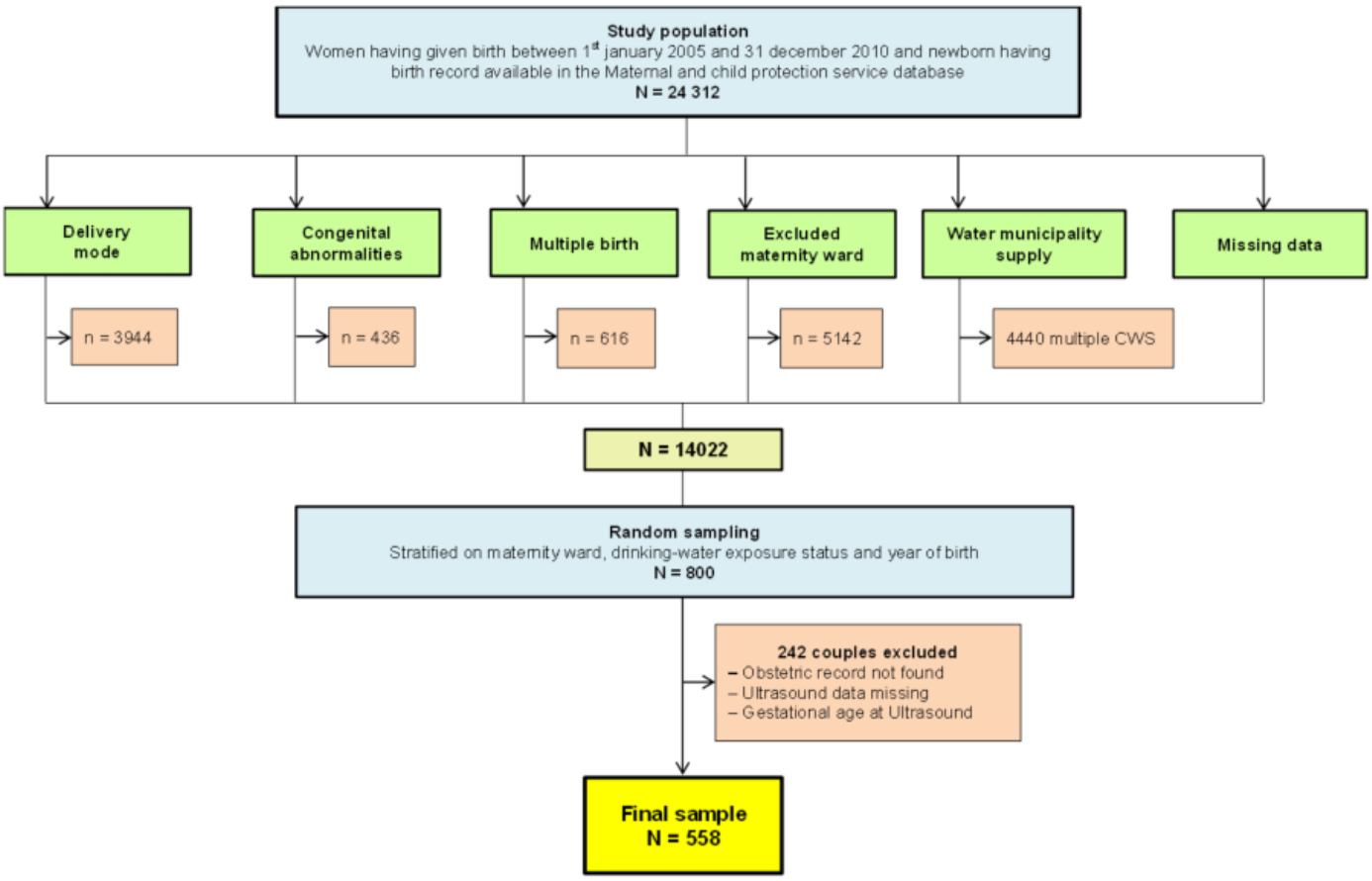

Figure 1

Flow chart, Deux-Sèvres, France. (CWS: Community water system)