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Drinking-water exposure to a mixture of nitrate and low-dose atrazine metabolites and small-for-gestational age (SGA) babies: A historic cohort study

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ABSTRACT

Background: Groundwater, surface water and drinking water are contaminated by nitrates and atrazine, an herbicide. They are present as a mixture in drinking water and with their endocrine-disrupting activity, they may alter fetal growth.

Objectives: To study an association between drinking-water atrazine metabolites/nitrate mixture exposure and small-for-gestational-age (SGA).

Methods: A historic cohort study based on birth records and drinking-water nitrate and pesticide measurements in Deux-Sèvres (France) between 2005 and 2009 was carried out. Exposure to drinking-water atrazine metabolites/nitrate mixture was divided into 6 classes according to the presence or absence of atrazine metabolites and to terciles of nitrate concentrations in each trimester of pregnancy. Regression analysis of SGA by mixture exposure at second trimester was subsequently conducted.

Results: We included 11,446 woman-neonate couples of whom 37.0% were exposed to pesticides, while 99.9% of the women were exposed to nitrates. Average nitrate concentration was from 0 to 63.30 mg/L. In the second trimester of pregnancy, the risk of SGA was different with mixture exposure when drinking-water atrazine metabolites, mainly 2hydroxyatrazine and desethylatrazine, were present and nitrate dose exposure increased: compared to single first tercile of nitrate concentration exposure, single second tercile exposure OR was 1.74 CI 95% [1.10; 2.75] and atrazine metabolites presence in the third tercile of nitrate concentration exposure OR was 0.87 CI 95% [0.45; 1.67].

Conclusions: It is possible that the association found at the second trimester of exposure with regard to birth weight may likewise be observed before birth, with regard to the estimated fetal weight, and that it might change in the event that the atrazine metabolites dose were higher or the nitrate dose lower. It would appear necessary to further explore the variability of effects.

1. Introduction

Nitrates (NO^{3-}) and nitrites (NO^{2-}) are water-soluble ions that are consequently often found in groundwater. Human activities (use of fertilizers and intensive agriculture) are the main causes of their presence in water. In the 2004–2007 period, in groundwater, of the 27 monitoring stations in the European Union, two thirds had average nitrate concentrations below 25 mg/L and only 15% above 50 mg/L (UE, 2011). In surface water, 21% of the stations had average nitrate concentrations below 2 mg/L, 37% in the range 2 to 10 mg/L, 3% in the range 40 to 50 mg/L and 3% above 50 mg/L (UE, 2011). For both types of water, several areas in France showed high concentrations in surface water (> 40 mg/L), and one of them was Poitou-Charentes (French Republic, 2011). Nitrates in drinking-water are associated with adverse human reproductive and developmental effects (spontaneous abortions, intrauterine growth restriction, birth defects such as neural tube defects, cardiac defects and anencephaly) (Manassaram et al., 2006). However, the maximum contaminant limit is defined on the basis of methemoglobinemia cases and was set by the World Health Organization at 50 mg/L (WHO, 2007).

Waters are also contaminated by pesticides. Most noncompliance with groundwater quality standards pertaining to pesticides is due to the main metabolite of atrazine, desethylatrazine, and to

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a lesser extent to atrazine itself, which is a triazine herbicide. Indeed, with regard to the 1827 French stations examined in 2007, the non-conformity rate was in descending order: 11% for desethylatrazine, 3% for atrazine, 1% for Aminomethylphosphonic acid (AMPA), 1% for glyphosate, 1% for bentazone (French Ecological Ministry, 2010).

In surface water, the main quantified pesticides are AMPA (43%), diuron (24%), glyphosate (22%) desethylatrazine (21%) and atrazine (14%). While atrazine has been banned from use since October 2003 (French Ecological Ministry, 2010), its presence in surface water may be explained by its long-lasting persistence in the environment (Jablonowski et al., 2011). Drinking-water limit value for pesticides is 0.10 μg/L for each pesticide and 0.50 μg/L for total pesticides (French Republic, 1998).

As Guillette et al. have demonstrated in their studies on alligators, nitrates are potential endocrine-disrupting contaminants. Since they are anti-androgenic (Guillette, 2006; Guillette and Edwards, 2005), they alter endocrine function. Indeed, nitrites and nitrates exert an inhibitory action on steroid hormone syntheses via conversion to nitric oxide. Nitric oxide inhibits the cytochrome P450 enzymes stopping the transformation of free cholesterol into progesterone (Panesar and Chan, 2000).

Some pesticides are likewise potentially endocrine-disrupting. Atrazine, for instance, is an androgen inhibitor with a weak estrogenic effect (McKinlay et al., 2008).

Birth weight depends on a wide variety of factors some of which are genetic and constitutional, demographic and psychosocial, obstetric or nutritional. Maternal morbidity during pregnancy, toxic exposure and antenatal care should also be taken into account (Kramer, 1987). Moreover, fetal growth depends on hormonal factors involving complex multifactorial relationships between growth factors (IGF-I) and steroid hormone expression via different receptors: estrogen receptors (ERα, ERβ) and progesterone receptor (PR) (Akram et al., 2011). Given their endocrine-disrupting activity and their capability of going through the placenta (Bruning-Fann and Kaneene, 1993; Rayner et al., 2007), atrazine metabolites and nitrates are indeed likely to affect fetal growth.

Only a few epidemiological studies on the effect of nitrate exposure on birth weight have been published to date (Bukowski et al., 2001; Manassaram et al., 2006; Ward et al., 2005), while studies dealing with pesticide exposure are pronouncedly more numerous (Burdorf et al., 2010; Cupul-Uicab et al., 2010; Konishi et al., 2009; Wang et al., 2011; Wigle et al., 2009; Wolff et al., 2007).

Bukowski et al. (2001) conducted a case-control ($n=210$ and 4098) study on singleton births in 1991–1994 in Canada aimed at assessing the impact of groundwater nitrate exposure on growth restriction (<2500 g). Exposure was defined in terms of median nitrate exposure in the groundwater supplying the mothers' residential address at the time of delivery. A significant relation between higher nitrate exposure (≥ 14 mg/L) and growth restriction was found in comparison with lower nitrate exposure (<14 mg/L): OR 2.40 CI 95% [1.75–3.27].

Only four pesticide studies have focused on atrazine (Chevrier et al., 2011; Munger et al., 1997; Ochoa-Acuna et al., 2009; Villanueva et al., 2005). All of them have found an association between drinking-water atrazine concentrations and fetal growth during different periods of exposure (entire pregnancy or only third trimester) but definitions of outcome differ from one study to the next (low birth weight, below 2500 g, SGA or fetal growth restriction) and the strength of association is small (Chevrier et al., 2011; Ochoa-Acuna et al., 2009; Villanueva et al., 2005).

While these studies have highlighted the effects on health of a single compound (either pesticides or nitrates), most of them have not examined the consequences of cumulative exposure to a mixture of pesticides and nitrates (Mnif et al., 2011). On rats, birth weight increases with a mixture containing atrazine while it decreases when atrazine is on its own (Enoch et al., 2007). On amphibians, modification of sex ratio following exposure to a mixture of atrazine and nitrates is higher than with atrazine or nitrates on their own (Orton et al., 2006). On humans, the risk of childhood cancers is different with a mixture of pesticides and nitrates than is the case with isolated chemicals (OR 1.18 CI 95% [0.63;2.21] for mixture exposure vs OR 1.10 CI 95% [0.78;1.56] for atrazine exposure and OR 1.49 CI 95% [1.22;2.83]) for nitrate exposure (Thorpe and Shirmohammadi, 2005).

A recent study on 112 full-term neonates showed a reduced birth weight related to pesticide mixtures detected in cord blood (Wickerham et al., 2012). However, to our knowledge there exists no epidemiological study of a possible association of exposure with a mixture of different classes of chemicals such as nitrates (natural inorganic compounds) and pesticides (phytosanitary products) in drinking water and SGA. With this in mind, our study is aimed at measuring the association of exposure with regard to a mixture of atrazine metabolites and nitrates in drinking water, and the occurrence of SGA.

2. Methods

The historic cohort study was carried out in Deux-Sèvres between 2005 and 2009. Deux-Sèvres is a district of Poitou-Charentes in western France with an area of 5999 km^2. Its population (362,944 inhabitants in 2007) resides in 305 municipalities. Agricultural activity is predominant and essentially involves livestock, predominantly sheep and goats, along with cereal production.

This study was based on measurements of drinking-water nitrates and pesticides and birth records. A French regional health agency (Agence Régionale de Santé) regularly assesses nitrates and 17 molecules of pesticide concentrations in drinking water: 2Hydroxyatrazine, Desethylatrazine, Isoproturon, Glyphosate, Atrazine, Bentazone, Linuron, Deisopropylatrazine, AMPA, Mecoprop, 4-Chloro-2-methylphenoxyacetic Acid (MCPA), Metolachlor, Chlortoluron, Simazine, Terbuthylazine, Desethylterbuthylazine and Dimethenamid. The number of pesticide and nitrate measurements by municipality is proportional, as required by law, to size of population. Municipalities are split or grouped into community water systems (CWS). Water quality is considered to be homogeneous within each CWS. All of the water samples ($n=1180$) were extracted from 65 different water treatment plants and CWS facilities between April 1st 2004 and December 31st 2009 so as to obtain complete exposure data throughout the pregnancy of each woman included in the study. In order to measure pesticide values, 1180 samples were taken: 342 (29%) in fall, 220 (17%) in winter, 323 (27%) in spring and 295 (25%) in summer. Among those 1180 samples, 1158 (98%) were extracted from treatment plant water, with an average of 22.5 ± 5.2 samples per station (from 1 to 34 samples), 22 (2%) samples were taken in community water systems, with an average of 13.4 ± 6.9 samples per station (from 1 to 17 samples). In order to measure nitrate values, 5926 samples were extracted: 1550 (26%) in fall, 1272 (21%) in winter, 1516 (26%) in spring and 1588 (27%) in summer. All samples came from community water systems, with an average of 186.6 ± 206.6 samples per station (from 18 to 620 samples).

Birth records came from the district office of maternal and childhood protection, via the mandatory infant health certificates completed by the hospital prior to an infant's discharge; all births are included. The available information indicates sex, weight at birth, gestational age (weeks of amenorrhea, reported by obstetrical staff at birth), age of mother, number of previous pregnancies, quality of pregnancy follow-up, smoking during pregnancy, parental occupations, single-parent family, medical history of SGA in siblings, gestational diabetes and place of residence at birth. Validation of the data drawn from birth records was carried out according to a methodology approved by the "Direction de la recherche, des études, de l'évaluation et des statistiques" (Collet and Vilain, 2010).

We identified all live births in Deux-Sèvres from January 1st 2005 through December 31st 2009 of neonates whose mother lived in the district at the time of birth and whose birth certificate had been recorded ($n=20,270$). We excluded from our analysis the non-environmental causes known to induce low birth weight or SGA such as multiple births, early deaths (before birth record completion), newborns with congenital malformations and births by cesarean section. On the basis of gestational age and birth date, we retrospectively calculated conception dates and each trimester of pregnancy. As there are large seasonal differences in pesticide usage and nitrate contamination, we identified for each trimester the season when the second trimester had occurred (if more than half of the trimester occurred in this season).

We also excluded 42 of the 305 municipalities in Deux-Sèvres because they had more than one CWS providing drinking water within the municipality or because no birth occurred in the municipality during the study. Sampling date, sampling location and CWS name were available for each measurement. Maternal place of residence on the date of birth was specified in view of attributing individual nitrate and/or pesticide exposure levels.

We merged the exposure variables with the individual data by place of residence. During a given trimester of pregnancy, each mother-neonate couple had several samples taken and for each sample, 17 different pesticides were analyzed. For each pesticide, we calculated the mean pesticide concentration for each trimester. When the mean was above the LQ, pesticide exposure was defined as positive for the trimester.

So as to define exposure, we used mean measurements for each trimester of pregnancy for: (1) atrazine metabolites or nitrates on their own and (2) a mixture of atrazine metabolites and nitrates. Exposure to a mixture of atrazine metabolites and nitrates was defined by positive atrazine metabolites exposure and in terms of the terciles of mean nitrate concentrations. Nitrate concentrations terciles were constructed in two steps: for each trimester, since the pregnant women had had several samples extracted in their CWS, we calculated the mean nitrate concentration for all samples taken during the trimester. Following that, we described the distribution and categorized it in terciles.

We defined six classes: unexposed to atrazine metabolites but exposed to the first tercile of mean nitrate concentrations, unexposed to atrazine metabolites but exposed to the second tercile of mean nitrate concentrations, unexposed to atrazine metabolites but exposed to the third tercile of mean nitrate concentrations, exposed to atrazine metabolites and to the first tercile of mean nitrate concentrations, exposed to atrazine metabolites and to the second tercile of mean nitrate concentrations, exposed to atrazine metabolites and to the third tercile of mean nitrate concentrations. The first tercile of nitrates included women whose average nitrate concentrations were below the quantification limit.

We defined household occupation according to the more advantageous occupation of either of the parents: disadvantageous (workers and unemployed), moderately advantageous (self-employed, employees and farmers) or advantageous (managers and executives) (Liberatos et al., 1988).

We defined SGA status as birth weight below the 10th centile for sex and gestational age, based on population growth curves obtained from 100,176 births in different French regions between 1984 and 1988 (Mamelle et al., 1996).

We calculated odds ratios (*OR*) and 95% confidence intervals (*CI*) of SGA using logistic regression and adjusting for household occupation, number of previous pregnancies, maternal age, medical history of low birth weight in siblings and smoking during pregnancy (Kramer, 1987). Analyses were conducted with SAS (version 9.3; SAS Institute, Cary, NC, USA®).

3. Results

The study population comprised 11,446 births in 263 municipalities. The selection of the study population, based on medical criteria (delivery mode, congenital malformations, multiple birth, early death of the newborn), type of water supply, presence of missing data and availability of drinking-water pesticide measurements is presented in Fig. 1.

Among the 11,446 woman/neonate couples the average number of pesticide measurements during pregnancy was 3 ± 1 per couple (3% with one pesticide measurement, 19% with two, 56% with three, 19% with four and 4% with five or more) and 39 ± 30 nitrate measurements per couple. 2748 (24%) were exposed to 2hydroxyatrazine and 1519 (13%) to desethylatrazine (Table1).

Study population characteristics are presented in Table 2.

During each trimester, average drinking-water positive atrazine metabolites measurements ranged from 0 to 0.1 μg/L for desethylatrazine and 2hydroxyatrazine. Among women who showed measurements of atrazine metabolites and/or nitrates, 4287 (37%) were exposed to atrazine metabolites at one of the three trimesters, 2293 (24%) during the first trimester, 2345 (25%) during the second trimester and 2174 (24%) during the third trimester. The second trimester occurred in 2759 cases (24%) in fall, in 2820 (25%) in summer, in 2933 (26%) in winter and in 2934 (26%) in spring.

During the entire pregnancy, almost all (99.9%) of the mothers were exposed to nitrates. The average nitrate concentrations ranged from 0 to 51.67 mg/L for first trimester, from 0 to 63.30 mg/L for second and third trimester.

Limits of the drinking-water nitrate concentration terciles were: 14.98 and 27.33 mg/L for the entire pregnancy; 14.12 and 26.67 mg/L for the first trimester, 14.13 and 26.99 mg/L for the second trimester and 14.50 and 27.30 mg/L for the third trimester.

During the entire pregnancy, average nitrate concentrations were 17.48 ± 12.99 mg/L for women exposed to atrazine metabolites and 22.91 ± 9.48 mg/L for women unexposed to atrazine metabolites.

Of the 11,446 births analyzed, 985 (8.6%) were SGA. Mean birth weight was 3310 ± 462 g and mean gestational age was 39 ± 1 weeks.

Accordingly with the literature, SGA prevalence increased in single-parent families, when the occupational group of the household was low, when mothers were primiparous, of extreme age and smoked during pregnancy, but it was not associated with rural location of residence (data available to the authors). Moreover, SGA rate was associated with the season when the second trimester of pregnancy occured: 7% in summer vs. 9% in spring vs. 9% in winter vs. 9% in fall, $p < 0.0066$.

Before proceeding to adjustments on the available confounders, we found an association between the six classes of mixture exposure and SGA status for one of the three trimesters and at the second trimester (Table 3).

At the second trimester, exposure to tercile 2 of nitrates without atrazine metabolites exposure significantly increased the risk of SGA (OR 1.74, CI 95% [1.10;2.75]) and mixture exposure significantly decreased the risk of SGA (Table 3). Adding the season when the second trimester had occurred in the model did not change this results.

4. Discussion

We have found that the risk of SGA at the second trimester of pregnancy was different with regard to exposure to chemical mixtures that is to say when exposure to drinking-water atrazine metabolite, mainly 2hydroxyatrazine and desethylatrazine, took place, and when nitrate dose exposure increased.

We have also found that, as has been regularly shown in Deux-Sèvres (GRAP, 2007) 2hydroxyatrazine and desethylatrazine were the most prevalent pesticides. This finding may be explained by a percentage of surface water higher than that of other French districts (66% vs 17%) and it is well known that pesticides come mainly from surface water (French Ecological Ministry, 2010).

We likewise found that since Deux-Sèvres is a department known to be vulnerable with regard to nitrates (French Republic, 2011), almost all mothers were exposed to the latter. As a result, study of drinking-water endocrine exposure with a single ED compound does not reflect actual conditions.

Endocrine Disruptor (ED) effect is an important challenge to traditional approaches in regulatory toxicology (Vandenberg et al., 2012). Indeed, ED effect is possible at low dose: biological changes occur in the range of typical human exposure or at doses below those tested in traditional toxicology assessments (Vandenberg et al., 2012). Infinitesimally low levels of exposure may cause endocrine or reproductive abnormalities, particularly if exposure occurs during a critical developmental window (Sheehan et al., 1999). Moreover, low doses may even exert more potent effects than higher doses (Vom Saal et al., 2007). In this 'nontraditional dose-response dynamics', the endocrine disruptor dose-response curve is often a U or inverted U shape (Vom Saal et al., 2007). Thus, a stimulation can happen at low dose whereas an inhibition will appear at high dose (Calabrese, 2002; Diamanti-Kandarakis et al., 2009).

In agreement with the literature (Bukowski et al., 2001), we found a positive and significant risk of SGA in exposure to single nitrates.

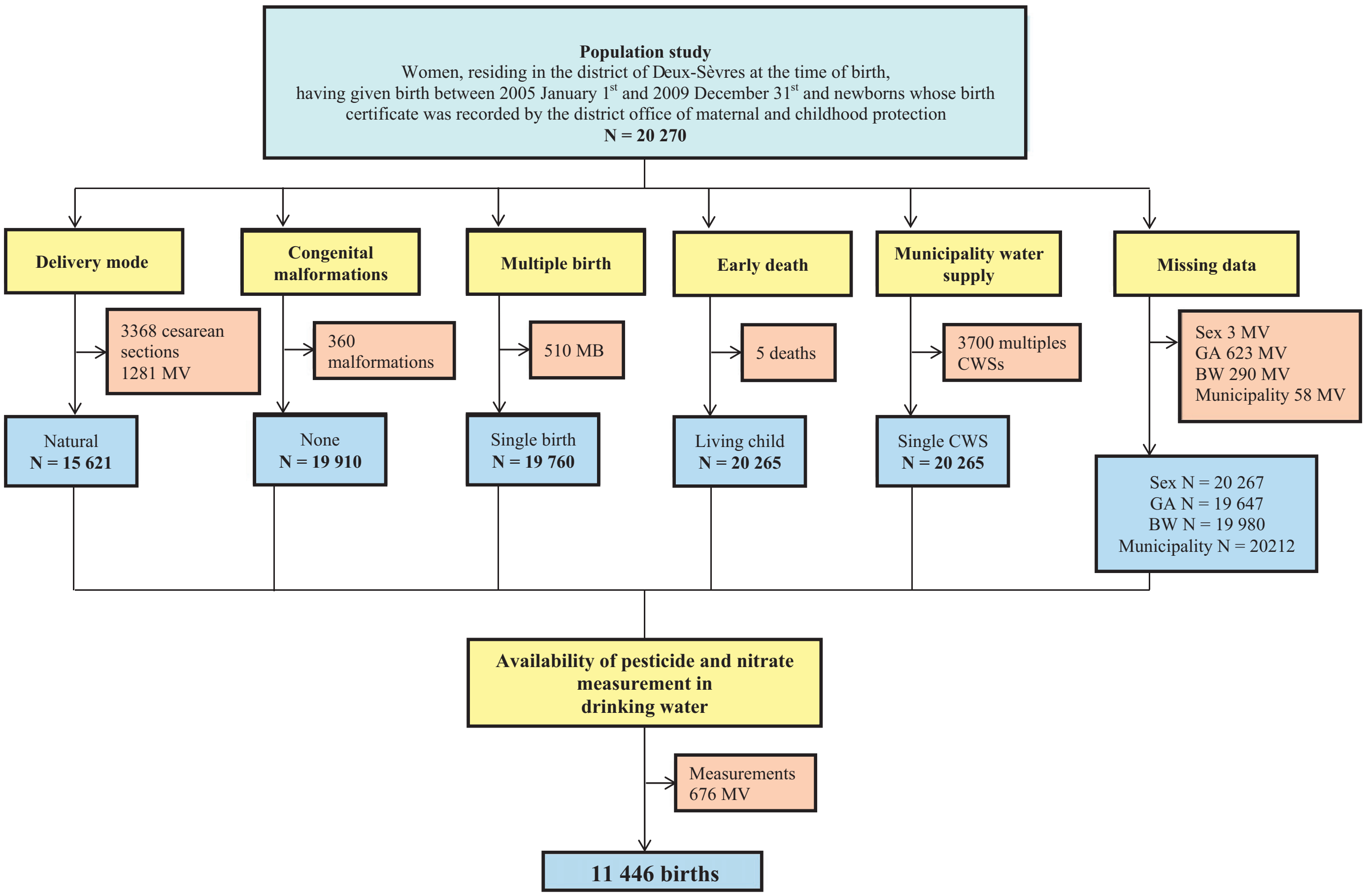

Fig. 1. Flow chart, Deux-Sèvres, France. (MV: missing values; MB: multiple births; CWS: community water system; GA: gestational age; BW: birth weight).

Table 1
Pesticide measurements 2005–2009, Deux-Sèvres, France.

	Measurement above quantification threshold		Measurement below quantification threshold	
	N	%	*N*	%
Number of mother/infant couples with pesticide measurements				
2Hydroxyatrazine	2748	24	8698	76
Desethylatrazine	1519	13	9927	87
Isoproturon	58	1	11,338	99
Glyphosate	33	0	11,413	100
Atrazine	30	0	11,416	100
Bentazone	17	0	11,429	100
Linuron	1	0	11,445	100
Deisopropylatrazine	0	0	11,446	100
Aminomethylphosphonic acid (AMPA)	0	0	11,446	100
Mecoprop	0	0	11,446	100
4-Chloro-2-methylphenoxyacetic Acid (MCPA)	0	0	11,446	100
Metolachlor	0	0	11,446	100
Chlortoluron	0	0	11,446	100
Simazine	0	0	11,446	100
Terbuthylazine	0	0	11,446	100
Desethylterbuthylazine	0	0	11,446	100
Dimethenamid	0	0	11,446	100

We found a positive but not significant risk of SGA in exposure to a mixture of atrazine metabolites and nitrates with a lower dose of nitrates. The mixture mechanism could be the result of similar modes of additive (anti-androgenic) action of atrazine and nitrates, which entail increased risk of SGA. Indeed, the concept of additivity assumes that chemicals act similarly, and that their modes of action result in dose or effect addition (Silins and Hogberg, 2011).

Moreover, we found a negative but not significant risk of SGA in exposure to a mixture of atrazine metabolites and nitrates at a higher dose of nitrates. This result may be linked to the concept of interaction, which assumes that a single chemical affects another chemical toxicity by synergism or antagonism, which depend on the dose and dose ratio (Silins and Hogberg, 2011). Indeed, a study of the effects of a binary mixture of four herbicides (atrazine, simazine, terbuthylanine, metolachlor) on microalgae growth rate showed differing effects (synergism or antagonism) that depended on each herbicide's mode of action (Perez et al., 2011) and another study showed an endocrine-disrupting effect on frogs after exposure to a mixture of atrazine and nitrates that varied according to the dose: antagonist effect (lesser snout-vent length) when nitrate concentrations are higher than atrazine concentrations and synergistic effects (greater snout-vent length) when atrazine concentrations are higher than nitrate concentrations (Sullivan and Spence, 2003).

Having added atrazine metabolites to our exposure variable, we found that SGA risk differed from that which was found without atrazine metabolites. Prior epidemiologic or experimental studies have nonetheless shown a positive association between atrazine exposure and SGA (Munger et al., 1997; Ochoa-Acuna et al., 2009; Powell et al., 2011; Rohr and McCoy, 2011; Davis et al., 2011) or one of its metabolites (Chevrier et al., 2011). However, the experimental studies used exposure concentrations above the environmental level and the epidemiologic studies have shown effects at first trimester (Chevrier et al., 2011), or third trimester (Ochoa-Acuna et al., 2009) but not at second trimester. Only Villanueva

Table 2
Characteristics of study population and potential confounders, Deux-Sèvres, France.

	Missing values		Total (N=11,446)	
	N	%	*N*	%
Sex of the newborn	0	0		
Boy			5856	51
Single-parent family	0	0		
Yes			576	5
Household occupation	51	1		
Disadvantageous			1729	15
Moderately advantageous			7078	62
Advantageous			2588	23
Rural location of residence	0	0		
Yes			3300	29
No			8146	71
Primiparity	691	6		
Yes			4422	41
Maternal age (years)	17	1		
< 26			2833	25
(26–29)			3527	31
(29–33)			2891	25
> 33			2178	19
History of low birth weight	4731	41		
Yes			233	4
Smoking during pregnancy	6361	56		
None			4137	81
1–10 cigarettes per day			790	16
> 10 cigarettes per day			158	3
Pregnancy illness	0	0		
Yes			534	5
Gestational diabetes	258	2		
Yes			173	2
Quality of pregnancy follow-up	2937	26		
Low			340	4
Drinking-water exposure to atrazine metabolites and nitrates mixture during second trimester*	1885	1		
Atrazine metabolites: no and Nitrates: tercile 1			1642	17
Atrazine metabolites: no and Nitrates: tercile 2			2835	30
Atrazine metabolites: no and Nitrates: tercile 3			2739	29
Atrazine metabolites: yes and Nitrates: tercile 1			1375	14
Atrazine metabolites: yes and Nitrates: tercile 2			351	4
Atrazine metabolites: yes and Nitrates: tercile 3			619	6

* Limits of the terciles were 14.13 and 26.99 mg/L.

et al. (2005) have focused on the second trimester and month of occurrence, and their results showed a negative but not significant association with SGA: OR 0.82 CI 95% [0.63–1.06] when the second trimester occurred in the May–September period in comparison with the October–April period. In point of fact, there exist critical periods of fetal development, and the effects of chemical influences will change according to the timing of exposure (Casals-Casas and Desvergne, 2011; Grandjean and Weihe, 2008). That much said, the effects of endocrine disruptors on fetal growth when exposure occurs during the second trimester have scarcely been studied (Barrett, 2009).

Our results with only two different endocrine disruptors are probably not representative of actual observational effects on human health, the reason being that we had not studied other endocrine disruptors such as drugs or bisphenol A, which are not routinely analyzed by the national French health agency.

The limits of our analysis were similar to those identified in previously published studies (Munger et al., 1997; Ochoa-Acuna et al., 2009; Villanueva et al., 2005). Firstly, birth record data were limited but enabled all births to be included and following data validation, only alcohol consumption was not used in our study.

Secondly, other sources of atrazine exposure such as air and food were not taken into account. However, it has been shown that atrazine metabolites, which are particularly frequent in our study, are rarely found in foods and that air concentrations, usually just above the detection limit, less than 0.30 μg/m^3 (Registry, 2003).

Thirdly, only the residence address at birth could have been recorded and residential mobility during pregnancy could not have been identified, so that in this study, misclassification bias is not to be excluded. However, residential mobility is usually moderate during pregnancy: from 9% in UK to 17% in USA (Chen L. et al., 2010; Fell et al., 2004; Hodgson et al., 2009) and the majority of women who move stay in the vicinity of their previous residence (Fell et al., 2004): the median distance between the two residences is only 1.4 km (Hodgson et al., 2009).

Fourthly, we assumed that the pregnant women studied drank only home tap water. The ratio of tap water intake versus bottled water intake was unknown in our study but in other studies, pregnant women's tap water intake was predominant: from 71% (Forssen et al., 2007) to 84% of all fluid intake consisted in home tap water (Smith et al., 2009). Zender et al. (2001) showed no significant difference in tap-water intake between 71 pregnant women and 43 non-pregnant women from Colorado: 75% versus 72% of all fluid intake (Zender et al., 2001). Moreover, the rate of home tap-water intake versus tap-water intake at other locations was unknown in our study but home tap-water consumption generally remains predominant: 53% (Zender et al., 2001), 63% (Kaur et al., 2004), 71% (Smith et al., 2009).

Fifthly, SGA was defined by a non-customized chart. It does not take into account constitutional factors linked to growth such as anthropometric parental characteristics that are linked to fetal biometrics (Albouy-Llaty et al., 2011). Only Chevrier et al. 2011 have taken into account these factors by using an expected birth weight model that reduces the number of SGA false positives. Information bias cannot be excluded in our study and prospective cohort design, which reduces these biases, remains the most reliable means of evaluating association between exposure to water pollutants and child outcome.

Lastly, while a definition of exposure to atrazine metabolites employing a quantification limit is of critical importance, laboratory analyses of water quality data do not require a detection limit in order to conclude that concentrations are above the regulatory limit. In our study, the data situated between detection and quantification limits were treated as negative exposure to atrazine metabolites, and it is possible that we have underestimated exposure and overestimated the relationship between exposure to atrazine metabolites and SGA.

Exposure assessment can be rendered more precise through exposure biomarkers such as blood and urine or cord blood, amniotic fluid, meconium and breast milk, with concentrations reflecting fetal exposure (Arbuckle, 2010). Barr et al. 2007 focused on atrazine and its urinary metabolite biomarker. However, atrazine is lipophilic and accumulates in mammary tissues according to the amount of the dose administered to rats. In fact, atrazine is transferred to the offspring via milk after an acute dose (Stoker and Cooper, 2007), so the biomarker for atrazine metabolites in breast milk should be investigated. Globally, it would appear necessary that studies develop biomarkers capturing cumulative exposure to all endocrine disruptors (Kortenkamp et al., 2007; Silins and Hogberg, 2011).

5. Conclusions

We found a positive association between exposure to drinking-water nitrates and SGA and a differing risk of SGA in the presence of nitrate and atrazine metabolites drinking-water mixtures, particularly when the dose of nitrates in the mixture increased at the second trimester. Our result shows that the effects of exposure to a mixture of atrazine metabolites and nitrates in drinking water have got to be studied further in order to better understand the mechanisms

Table 3
Association between exposure to atrazine metabolites nitrate mixture in drinking-water and prevalence of small-for-gestational age (SGA) babies during second trimester of pregnancy before and after adjustment for available confounders, Deux-Sèvres, France.

	Small for gestationnal age (SGA)							
	N SGA	% SGA	Unadjusted analysis			Adjusted analysis ($n=3346$)		
			OR	CI 95%	p	OR	CI 95%	p
Drinking-water exposure to atrazine metabolites and nitrates mixture during second trimester					0.002			0.050
Atrazine metabolites: no and Nitrates: tercile 1	120	7	1			1		
Atrazine metabolites: no and Nitrates: tercile 2	281	10	**1.40**	**[1.12;1.74]**		**1.74**	**[1.10; 2.75]**	
Atrazine metabolites: no and Nitrates: tercile 3	257	9	**1.29**	**[1.03;1.61]**		1.51	[0.96;2.40]	
Atrazine metabolites: yes and Nitrates: tercile 1	104	8	1.03	[0.78;1.35]		1.32	[0.74;2.35]	
Atrazine metabolites: yes and Nitrates: tercile 2	27	8	1.06	[0.68;1.63]		1.21	[0.65;2.25]	
Atrazine metabolites: yes and Nitrates: tercile 3	38	6	0.82	[0.57;1.20]		0.87	[0.45; 1.67]	
Household occupation					< 0.001			0.051
Disadvantageous	219	13	**2.19**	**[1.77;2.71]**		**1.73**	**[1.04;2.88]**	
Moderately advantageous	602	9	**1.40**	**[1.17;1.68]**		**1.65**	**[1.09;2.50]**	
Advantageous	161	6	1			1		
Maternal age (years)					< 0.001			0.681
< 26	319	8	**1.51**	**[1.28;1.79]**		1.01	[0.73;1.38]	
26 to < 29	273	7	1			1		
29 to < 33	211	11	0.94	[0.78;1.13]		0.99	[0.70;1.41]	
> 33	177	8	1.05	[0.87;1.28]		1.22	[0.85;1.74]	
History of low birth weight					< 0.001			< 0.001
Yes	596	20	**2.43**	**[1.74;3.39]**		**2.22**	**[1.43;3.44]**	
No	46	9	1			1		
Smoking during pregnancy					< 0.001			< 0.001
None	275	7	1			1		
1 to10 cigarettes per day	141	18	**3.05**	**[2.45;3.80]**		**2.66**	**[2.01;3.53]**	
> 10 cigarettes per day	36	23	**4.14**	**[2.80;6.13]**		**2.66**	**[1.53;4.64]**	

of mixture dose response at different times. Further epidemiologic studies based on ultrasound fetal weight estimates, preferentially with atrazine metabolites and nitrate biomarkers, would be required.

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