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## Atmospheric pollution and Human reproduction.

Lise Giorgis-Allemand

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## **THÈSE**

Pour obtenir le grade de

**DOCTEUR DE LA COMMUNAUTE UNIVERSITE  
GRENOBLE ALPES**

Spécialité : **Modèles, méthodes et algorithmes en biologie**

Arrêté ministériel : 7 août 2006

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U1209 et CNRS UMR 5309  
dans l'École Doctorale Ingénierie pour la Santé, la Cognition  
et l'Environnement

# **Pollution atmosphérique et reproduction humaine.**

# **Atmospheric pollution and human reproduction.**

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# Summary<sup>1</sup>

Remerciements .....	i
Summary .....	ii
List of Tables .....	iv
List of Figures .....	v
List of abbreviations .....	vi
<b>Chapter I: Introduction .....</b>	<b>2</b>
<b>I. French summary .....</b>	<b>4</b>
<b>II. Overview .....</b>	<b>5</b>
<b>III. Atmospheric pollution .....</b>	<b>7</b>
1. Sources of atmospheric pollutants .....	7
2. Regulation of atmospheric pollution .....	8
3. Temporal trends .....	9
4. Assessment of exposure to atmospheric pollutants in epidemiological studies .....	10
a. Nearest monitoring station network .....	10
b. Land Use Regression, dispersion and chemical transport models .....	11
c. Personal monitors .....	12
d. Estimating exposures during the right time period .....	14
<b>IV. Human Reproduction .....</b>	<b>15</b>
1. Overview .....	15
2. Definitions of the main fecundity and pregnancy related outcomes .....	15
a. Before fertilization .....	15
b. Between fertilization and pregnancy detection .....	17
c. After fertilization .....	19
3. Human reproduction and atmospheric pollution .....	22
a. Air pollution effect on gametogenesis .....	22
b. Air pollution effect on fecundity, fertility, and fecundability .....	22
c. Air pollution effect on birth weight, preterm delivery and stillbirth .....	25
4. Human reproduction and meteorological parameters .....	26
<b>V. Objectives of the thesis .....</b>	<b>29</b>
<b>Chapter II: Methods .....</b>	<b>32</b>
<b>I. French Summary .....</b>	<b>34</b>
<b>II. OBSEFF study .....</b>	<b>35</b>
1. Population .....	35
2. Outcomes definition and statistical analysis .....	36
a. Menstrual cycle characteristics .....	36
b. Time to pregnancy studies .....	37
3. Atmospheric pollution .....	40
a. Exposure model .....	40
b. Back-extrapolation and seasonalization .....	40
c. Exposure windows considered .....	42
4. Authors contributions .....	42
<b>III. ESCAPE study .....</b>	<b>43</b>
1. Population .....	43
2. Atmospheric pollution .....	44
a. Exposure model .....	44
b. Backextrapolation and seasonalization .....	44
c. Exposure windows considered .....	45
d. Residential mobility during pregnancy .....	45
3. Meteorological parameters .....	46
4. Preterm birth definition and statistical analysis .....	47
5. Authors contribution .....	47
<b>Chapter III: Before fertilization: Atmospheric pollution and menstrual cycle .....</b>	<b>50</b>
<b>I. French summary .....</b>	<b>52</b>
<b>II. Research letter, to be submitted to Epidemiology .....</b>	<b>54</b>
<b>III. eAppendix .....</b>	<b>59</b>

<sup>1</sup> Conformément aux consignes de l'école doctorale Edisce concernant les thèses rédigées en anglais, chaque chapitre est précédé d'un résumé en français.

<b>Chapter IV: Around fertilization: Effects of Atmospheric pollution on couples' fecundity .....</b>	<b>70</b>
<b>I. French summary .....</b>	<b>72</b>
<b>II. Article.....</b>	<b>73</b>
1. Abstract .....	76
2. Introduction .....	77
3. Methods .....	79
4. Results .....	83
5. Discussion .....	86
6. Conclusion.....	90
7. References .....	91
8. Tables and figures.....	94
<b>III. Supplemental material.....</b>	<b>102</b>
<b>Chapter V: After fertilization: Effects of Atmospheric pollution on preterm delivery .....</b>	<b>106</b>
<b>I. French summary .....</b>	<b>108</b>
<b>II. Article.....</b>	<b>110</b>
1. Abstract .....	114
2. Introduction .....	115
3. Methods .....	116
4. Results .....	119
5. Discussion .....	121
6. Conclusions .....	124
7. Acknowledgments .....	125
8. References .....	126
9. Tables and figures.....	130
<b>III. Supplement material.....</b>	<b>135</b>
<b>Chapter VI: Discussion.....</b>	<b>150</b>
<b>I. French summary .....</b>	<b>152</b>
<b>II. Main findings.....</b>	<b>153</b>
<b>III. Methodological issues .....</b>	<b>153</b>
1. Confounding .....	153
2. Outcomes assessment .....	154
a. Menstrual cycle .....	154
b. Fecundity.....	154
c. Preterm birth .....	155
3. Exposure assessment .....	156
a. Back-extrapolation and seasonalization .....	156
b. Geocoding .....	158
c. Residential mobility .....	158
d. Time space activity .....	159
4. Study design and statistical methods .....	160
a. Menstrual cycle study .....	160
b. Fecundity studies.....	161
c. Preterm delivery study .....	165
<b>IV. Plausibility of the findings.....</b>	<b>169</b>
1. Do air pollutants levels influence menstrual cycle length? .....	169
2. Do air pollutants levels influence fecundity? .....	170
3. Are the reported effects of air pollution on preterm delivery a statistical artefact?.....	171
<b>V. Conclusion .....</b>	<b>173</b>
<b>References.....</b>	<b>176</b>
<b>Annexes .....</b>	<b>194</b>
<b>I. Publications and communications .....</b>	<b>196</b>
<b>II. Comment from Ha and Mendola (American Journal of Epidemiology) .....</b>	<b>199</b>
<b>III. Reply to Ha and Mendola.....</b>	<b>200</b>

# List of Tables<sup>2</sup>

Table I-1: Air quality standards as defined in the EU Ambient Air Quality Directive and WHO Air Quality Guideline (AQG), adapted from (EU, 2015) .....	8
Table I-2: Comparison of various outdoor atmospheric pollution exposures assessment used in epidemiological studies .....	13
Table I-3: Publications on air pollution and fecundity-related outcomes in Human.....	24
Table I-4: Characteristics of the studies on preterm delivery and PM <sub>2.5</sub> exposure reviewed by Sun et al. (2015) .....	27
Table III-1: Adjusted change in the duration of cycle, follicular and luteal phase associated with atmospheric pollution levels (n=181).....	58
Table IV-1: Characteristics of the population included in current duration or prevalent cohort studies with defined CDUI or time to pregnancy. Note that 585 subjects of the prevalent cohort analysis are also included in the current duration analysis. ....	94
Table IV-2 Association between adjustment factors and time to pregnancy. Current duration and prevalent cohort approach. ....	96
Table IV-3: Atmospheric pollutants and time to pregnancy. Current duration and prevalent cohort approach. ....	97
Table IV-4: Atmospheric pollutants and time to pregnancy. Current duration and prevalent cohort approach, sensitivity analyses .....	98
Table V-1: Characteristics of the Study Population (N=71,493 Live Births from 13 European Cohorts part of ESCAPE project, 1994-2010). ....	130
Table V-2: Associations Between Atmospheric Pollutants and Preterm Birth (Pooled Analysis of 13 European Cohorts part of ESCAPE project, 1994-2010). ....	132
Table VI-1: Summary of the features of the study designs for human fecundity.....	163
Table VI-2: Possible bias in the study designs used to study fecundity and possible solutions. ....	164

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<sup>2</sup> Tables within supplemental material of articles are numbered separately and not included in this list.

# List of Figures<sup>3</sup>

Figure I-1: Evolution of emission of pollutants in mainland France with their sources between 2000 and 2014 (Commissariat général au développement durable, 2015) .....	7
Figure I-2: Percentage of the EU urban population potentially exposed to air pollution exceeding EU air quality standards, (EU, 2011) .....	9
Figure I-3: Evolution of SO <sub>2</sub> , NO <sub>2</sub> and PM <sub>10</sub> concentrations over 2000-2014 in France (Commissariat général au développement durable, 2015) .....	9
Figure I-4: Location of background stations measuring NO <sub>2</sub> and PM <sub>10</sub> in France between 2002 and 2009 .....	11
Figure I-5: Example of the use of GPS data with SIRANE dispersion model with a 10x10 m resolution for PM <sub>10</sub> in Grenoble, France .....	12
Figure I-6: Phases of the menstrual cycle .....	16
Figure I-7: gestational age, preterm delivery and pregnancy losses .....	21
Figure I-8: overview of the timeline of reproduction-related outcomes (adapted from Slama, 2014).....	29
Figure II-1: urinary level of a progesterone metabolite (pregnanediol-3-alpha-glucuronide, PdG) for one study participant .....	36
Figure II-2: Exposures windows and study design, OBSEFF study.....	39
Figure II-3: Map of cohorts included in the ESCAPE preterm delivery study.....	43
Figure IV-1: Study design, example for five women (A to E) .....	99
Figure IV-2: Flow chart .....	100
Figure IV-3: Locations of home addresses of the 544 women included in the current duration analysis with levels of NO <sub>2</sub> estimated in year 2009 with the air pollution model. ....	101
Figure V-1: Localization of the study areas within Europe and pollutants assessed at each location. The surface of the circle is proportional to the number of subjects in each center. European Cohorts part of ESCAPE project, 1994-2010. ....	133
Figure V-2: Preterm birth and A) Temperature (first trimester), B) Humidity (whole pregnancy; discrete time survival model) and C) Atmospheric pressure first trimester average..	134
Figure VI-1: Exposure to PM <sub>2.5</sub> during whole pregnancy and preterm delivery in studies using a survival model or a matched case-control. ....	171

<sup>3</sup> Figures within supplemental material of articles are numbered separately and not included in this list.



# List of abbreviations

CDUI: Current Duration of Unprotected Intercourse (outcome of the current duration approach)

CI: confidence interval

ESCAPE: European Study of Cohorts for Air Pollution Effects

EU: European Union

GIS: Geographic Information System

GPS: Global Positioning System

hCG: Human Chorionic Gonadotropin

HR: Hazard Ratio

ICSI: IntraCytoplasmic Sperm Injection

IGN: Institut National de l'information Géographique et forestière

IVF: In Vitro Fertilisation

INERIS: Institut National de l'EnviRonnement Industriel et des RisqueS

IQR: Interquartile Range

LH: luteinizing hormone

LMP: last menstrual period

LUR: Land Use Regression

NO<sub>2</sub>: nitrogen dioxide

NO<sub>x</sub>: nitrogen oxides

OBSEFF: OBServatoire de la Fertilité en France

OR: Odds Ratio

O<sub>3</sub>: ozone

PM: particulate matter

PM<sub>10</sub>: particulate matter with aerodynamical diameter of less than 10 µm

PM<sub>2.5</sub>: particulate matter with aerodynamical diameter of less than 2.5 µm

PM<sub>coarse</sub> or PM<sub>10-2.5</sub>: particulate matter with aerodynamical diameter between 2.5 µm and 10 µm

SO<sub>2</sub>: sulfur dioxide

TR: Time Ratio

TTP: Time To Pregnancy

WHO: World Health Organization





# Chapter I: Introduction



## I. French summary

L'impact de certaines expositions environnementales -en particulier la pollution atmosphérique- sur la santé humaine est bien documenté et établi. La pollution atmosphérique peut venir de sources naturelles (volcans) ou anthropiques (trafic routier, industries, chauffage domestique) et est composée d'une multitude de gaz et de particules. Une fraction importante de la population est exposée à la pollution atmosphérique ; ses effets sur la mortalité et la santé cardiovasculaire et respiratoire sont connus, et un effet de l'exposition durant la grossesse sur le poids de naissance ou la croissance fœtale est probable. En revanche, la capacité des couples à concevoir et les paramètres de la fertilité féminine ont été très peu étudiés. Pourtant, la question d'une association entre pollution atmosphérique et fertilité des couples mériterait d'être approfondie en raison des résultats des quelques études animales et humaines existantes. La reproduction est une succession d'étapes qui commencent in utero avec la formation de l'appareil reproducteur. Un effet délétère sur la reproduction pourrait se produire à différentes étapes, que ce soit antérieures (formation des gamètes) ou au cours de la grossesse (implantation de l'embryon, fonction cardio-vasculaire maternelle).

L'objectif de ce doctorat est de mieux documenter un effet éventuel de la pollution atmosphérique sur la fonction de reproduction humaine et tout particulièrement :

- 1) Avant la conception : étudier l'association entre la pollution atmosphérique et les caractéristiques du cycle menstruel,
- 2) Autour de la conception : étudier l'association entre la pollution atmosphérique et la probabilité de grossesse en France en utilisant deux designs d'études sur la même population
- 3) Le déroulement de la grossesse : étudier l'association entre la pollution atmosphérique et les naissances prématurées.

## II. Overview

In the last centuries, human populations have caused and faced numerous environmental, technological and societal modifications. Many of them are positive and save lives -the development of hygiene, the invention of antibiotics, wider access to clean water, electricity, health care, contraception and education. The implementation of these changes is not uniform within and between populations. In 1990, 76 % of the World population used improved drinking water source and this proportion increased to 91 % in 2015 (WHO and UNICEF, 2015). Life expectancy at birth increased by in average 6 years for children born in 2012 compared to those born in 1990 and this improvement has been seen across all country-income groups (WHO, 2014). Focusing on maternal and child health, globally, maternal mortality fell by 44 % between 1990 and 2015, but the number of maternal deaths was still estimated to be 303,000 in 2015 (WHO et al., 2015). Even if huge progresses have been done in the past decades, there still are margins of improvements and a large geographical heterogeneity. For example, 99 % of the maternal deaths occurring in 2015 happened in developing countries which include 90 % of the number of births worldwide. 66 % of the total number of maternal deaths occurred in Sub-Saharan Africa (26% of the number of birth worldwide) and 22% in Southern Asia (8% of the number of birth worldwide) (WHO et al., 2015)<sup>4</sup>.

Amongst all the pollutants generated by human activities, atmospheric pollution is one of the most studied. The London Smog of 1952, causing more than 3,000 deaths (Bell and Davis, 2001), has been a turning point in the study of the effects of atmospheric pollution on health. The most industrialized countries have taken measures to fight air pollution, but the problem in rapidly emerging and newly industrialized countries such as India and to some extent China remains. In 2012, an average level of PM<sub>10</sub> (particulate matter with aerodynamical diameter of less than 10 µm) of 229 µg/m<sup>3</sup> was measured in Delhi, India, while in 2013, an average level of PM<sub>10</sub> of 108 µg/m<sup>3</sup> was measured in Beijing, China, and of 141 µg/m<sup>3</sup> in Dakar, Senegal. These levels are far higher than the levels observed in Paris, France (average level of PM<sub>10</sub> measured in 2014 of 28 µg/m<sup>3</sup>) or in Ottawa, Canada (average level measured in 2013 of 11 µg/m<sup>3</sup>).<sup>5</sup> Thus, there still are margins of improvements and a large geographical heterogeneity for levels of atmospheric pollution too.

---

<sup>4</sup> Number of births worldwide estimated from “The State of the World’s Children 2016 Statistical Tables”, UNICEF, June 2016.

<sup>5</sup> Exposures levels from the Ambient Air Pollution Database, WHO, May 2016.

Atmospheric pollution can have various effects on human health at the short and long terms. WHO classified air pollution as carcinogenic for humans<sup>6</sup> and estimated that in 2012, 90% of the world population was exposed to levels of particulate matter higher than the WHO Air Quality Guidelines, and that about 3 million deaths were due to particulate air pollution (WHO, 2016). Mortality, hospital admission and impaired lung function (symptoms of bronchitis) increase with atmospheric pollution (Brunekreef and Holgate, 2002). Air pollution also affects cardiovascular system (Dockery, 2001; Du et al., 2016) and have acute effect on respiratory system (Goldizen et al., 2016). As we will see later, air pollution may also affect reproductive health.

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<sup>6</sup> IARC press release n°221, 17/10/2013: [http://www.iarc.fr/en/media-centre/pr/2013/pdfs/pr221\\_E.pdf](http://www.iarc.fr/en/media-centre/pr/2013/pdfs/pr221_E.pdf)



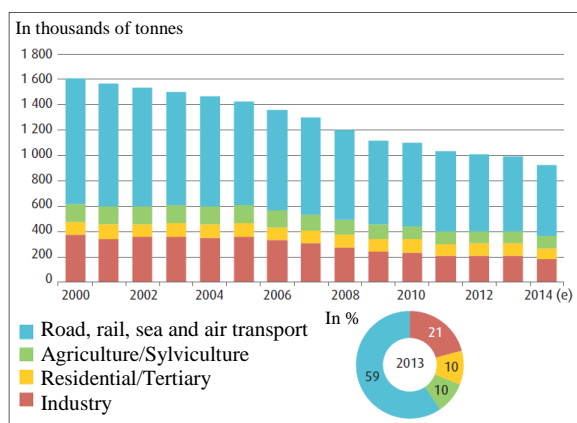
### III. Atmospheric pollution

#### 1. Sources of atmospheric pollutants

Air pollution is a complex mixture of gases and particulate matter emitted from various natural and anthropogenic sources (Figure I-1). Nitrogen oxides (NO<sub>x</sub>, nitrogen oxides, which include nitrogen dioxide, NO<sub>2</sub>) are mostly created by anthropogenic emission and particularly combustion of fossil fuels from stationary sources (industries, power plant, house heating) or mobile sources (cars and other vehicles). In an urban context, nitrogen oxides are often considered as a marker of traffic-related air pollution (Cyrus et al., 2012; Favarato et al., 2014; Hamra et al., 2015). Particulate matter (PM) composition include many organic and inorganic materials from natural sources, such as volcanos or seas, but also man-made sources, such as factories, motor vehicles emissions, construction activities...). The exposure in urban areas is mostly due to anthropogenic sources such as heating, traffic and industry. (EU, 2015; Yang and Omaye, 2009). In addition to chemical composition, particulate matter can be characterized by their aerodynamical diameter: up to 10 µm (PM<sub>10</sub>) or below (up to 2.5 µm: PM<sub>2.5</sub>, up to 1 µm: PM<sub>1</sub>, or between 2.5 and 10 µm: coarse fraction, PM<sub>10-2.5</sub>).

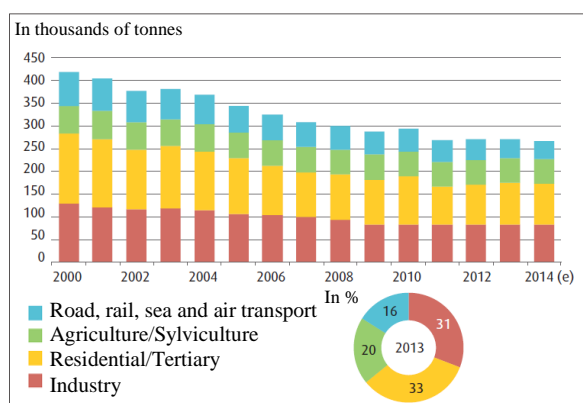
**Figure I-1: Evolution of emission of pollutants in mainland France with their sources between 2000 and 2014 (Commissariat général au développement durable, 2015)**

#### A) NO<sub>x</sub>



Source : Citepa, format Secten, mise à jour avril 2015

#### B) PM<sub>10</sub>



Source : Citepa, format Secten, mise à jour avril 2015

Meteorology is an important determinant of atmospheric pollution as it influences both emissions (more heating in cold days), dispersion -i.e. the transport of pollutants from their source- of pollutants (through wind and other weather conditions) and atmospheric chemical reactions (through sunlight for ozone). Anthropogenic emissions of NO<sub>2</sub> and PM are usually higher in the period of the year with the lowest temperatures due to heating sources (heating oil, wood). Wind influences long range transport of stable pollutants as for example black carbon -part of PM<sub>2.5</sub> obtained from an incomplete combustion- that can be observed as far as in Artic (Law and Stohl, 2007). Wind, temperature and solar radiation influence the ground and the atmospheric boundary layer (the part of the atmosphere that is influenced by the planetary surface, with a thickness about 1 km), generating turbulence mixing. When the atmospheric boundary layer is stable, an inversion layer can appear, corresponding to higher temperature at higher height. Then, the atmospheric convection that occurs in neutral and unstable atmospheric boundary layer stops, impacting dispersion and dilution of atmospheric pollution and leading to the formation of fogs (Cushman-Roisin, 2014; Sportisse, 2008).

## 2. Regulation of atmospheric pollution

Atmospheric pollution is regulated and measured in many places of the world. The World Health organization (WHO) has issued guidelines and in the European Union (EU), additional guidelines values are defined by the Ambient Air Quality Directive. Guidelines values exist in particular for particulate matter and nitrogen oxides (Table I-1), which will be the major pollutants developed in this manuscript. In France, air quality monitoring stations measure these pollutants on an hourly basis in urban areas with more than 100,000 inhabitants<sup>7</sup>.

**Table I-1: Air quality standards as defined in the EU Ambient Air Quality Directive and WHO Air Quality Guideline (AQG), adapted from (EU, 2015)**

Pollutant	Averaging period	EU Air Quality Directive			WHO AQG
		Objective and legal nature	Concentration	Comments	Guideline
PM <sub>10</sub>	1 day	Limit value	50 µg/m <sup>3</sup>	Not to be exceeded on more than 35 days per year	50 µg/m <sup>3</sup> <sup>(a)</sup>
PM <sub>10</sub>	Calendar year	Limit value	40 µg/m <sup>3</sup>		20 µg/m <sup>3</sup>
PM <sub>2.5</sub>	1 day	Limit value	25 µg/m <sup>3</sup>		25 µg/m <sup>3</sup> <sup>(a)</sup>
PM <sub>2.5</sub>	Calendar year				10 µg/m <sup>3</sup>
NO <sub>2</sub> , NO <sub>x</sub>	1 hour	Human health limit value	200 µg/m <sup>3</sup>	not to be exceeded on more than 18h per year	200 µg/m <sup>3</sup>
NO <sub>2</sub> , NO <sub>x</sub>	Calendar year	Human health limit value	40 µg/m <sup>3</sup>		40 µg/m <sup>3</sup>
NO <sub>2</sub> , NO <sub>x</sub>	1 hour	Alert <sup>(b)</sup> threshold	400 µg/m <sup>3</sup>		

<sup>(a)</sup> 99th percentile (3 days/year)

<sup>(b)</sup> To be measured over 3 consecutive hours at locations representative of air quality over at least 100 km<sup>2</sup> or an entire zone or agglomeration, whichever is the smaller.

Sources: EU, 2008; WHO, 2006a; WHO, 2008; EU 2015

<sup>7</sup> Code de l'environnement.

### 3. Temporal trends

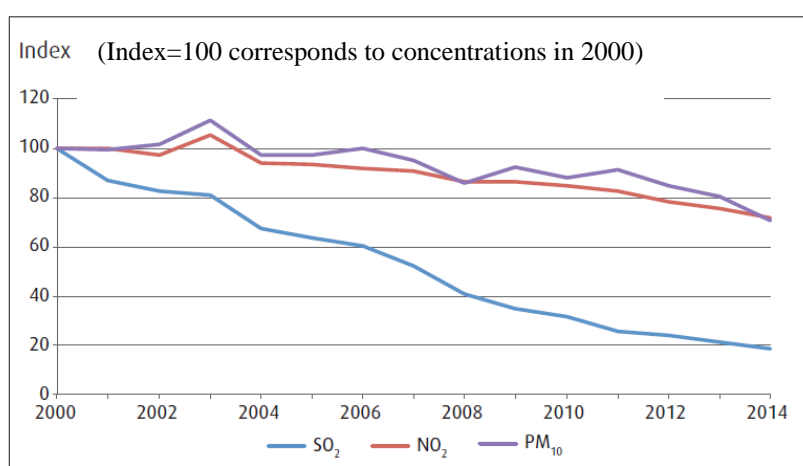
Since a few decades and with more and more stringent regulation, the environmental levels declined in Europe (EU, 2011) (Figure I-2) and in France (Figure I-3) for NO<sub>2</sub> and PM<sub>10</sub>. This improvement is not observed in all regions of the world; PM<sub>2.5</sub> annual concentrations decreased between 2008 and 2013 in high-income regions (Americas, Europe, Western Pacific) but increased in other regions (WHO, 2016).

**Figure I-2: Percentage of the EU urban population potentially exposed to air pollution exceeding EU air quality standards, (EU, 2011)**



Source: EEA, 2011c (CSI 004).

**Figure I-3: Evolution of SO<sub>2</sub>, NO<sub>2</sub> and PM<sub>10</sub> concentrations over 2000-2014 in France (Commissariat général au développement durable, 2015)**



Note: these indicators are based on an evolving sample of monitoring stations: selection of stations operating year  $n$  and year  $n-1$ . This method allows for the development of the monitoring network while retaining a constant field of coverage between two years.

Field of coverage: metropolitan France excluding Corsica.

Source: Géod'Air, May 2015. Processed by: SOeS, 2015

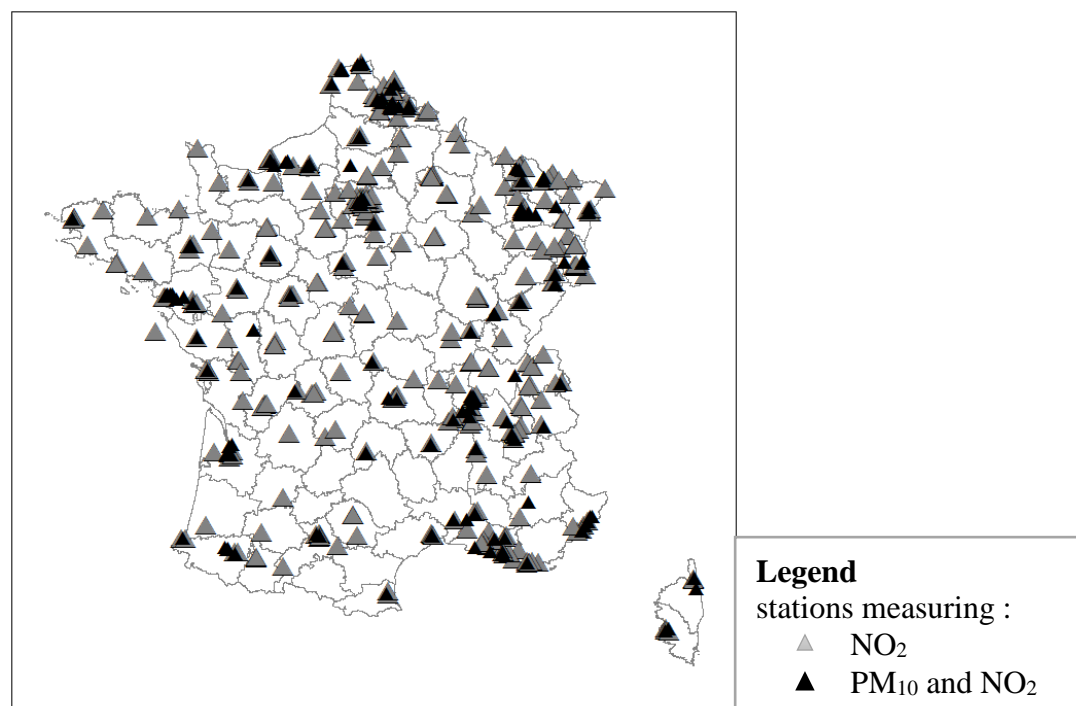
#### 4. Assessment of exposure to atmospheric pollutants in epidemiological studies

Various methods are used to estimate exposure to atmospheric pollution in environmental epidemiology. Table I-2 (page 13) summarizes these methods while the most frequently used ones will be described below.

##### *a. Nearest monitoring station network*

Many countries have a national network of air quality monitoring stations to measure regulated atmospheric pollutants outdoor levels; with stations mostly located in large urban areas or near industrial sites. Using data from monitoring network is often done to estimate exposure to atmospheric pollutants for participants in epidemiological studies as data can be accessed easily (example of studies: Chang et al., 2015; Faiz et al., 2012; Slama et al., 2013). The home address of each subject is generally assigned to the nearest background station functioning during the study period. As most of the monitoring stations are located in cities and as there are only a few ones in each city, the spatial resolution of this approach is quite low (see Figure I-4 for the spatial resolution in France). Monitoring stations data are generally representative of daily temporal variations of atmospheric pollution in a wide area, but only representative of the exposure levels in a small area around them. Regarding NO<sub>2</sub>, in a study conducted with passive samplers in four European cities, more between-site variation was observed than within-site and the spatial contrasts were stable from a measurement campaign to another (Lebret et al., 2000) and in Munich, Germany, the daily averages concentrations were correlated between 0.5 and 0.9 across the seven monitors (Slama et al., 2007). In 36 areas included in European Study of Cohorts for Air Pollution Effects (ESCAPE), there was a large spatial variability observed within study areas, with more variability in traffic than in background sites (Cyrus et al., 2012). Regarding PM, in 20 areas included in ESCAPE, significant within-area variations was observed for PM<sub>coarse</sub> and PM<sub>2.5</sub> absorbance compared to between-areas but not for PM<sub>10</sub> and PM<sub>2.5</sub>; although the within-area variations were smaller for those two pollutants, a clear spatial contrast within each area still existed between them too (Eeftens et al., 2012a). Daily concentrations of PM<sub>10</sub> between an urban (Vienna) and a rural station (Streithofen) separated by 30 km (Puxbaum et al., 2004) in Austria had a coefficient of correlation of 0.68 over a year, with higher correlation in summer (0.78) than winter (0.67) (Gomiscek et al., 2004).

**Figure I-4: Location of background stations measuring NO<sub>2</sub> and PM<sub>10</sub> in France between 2002 and 2009**



*b. Land Use Regression, dispersion and chemical transport models*

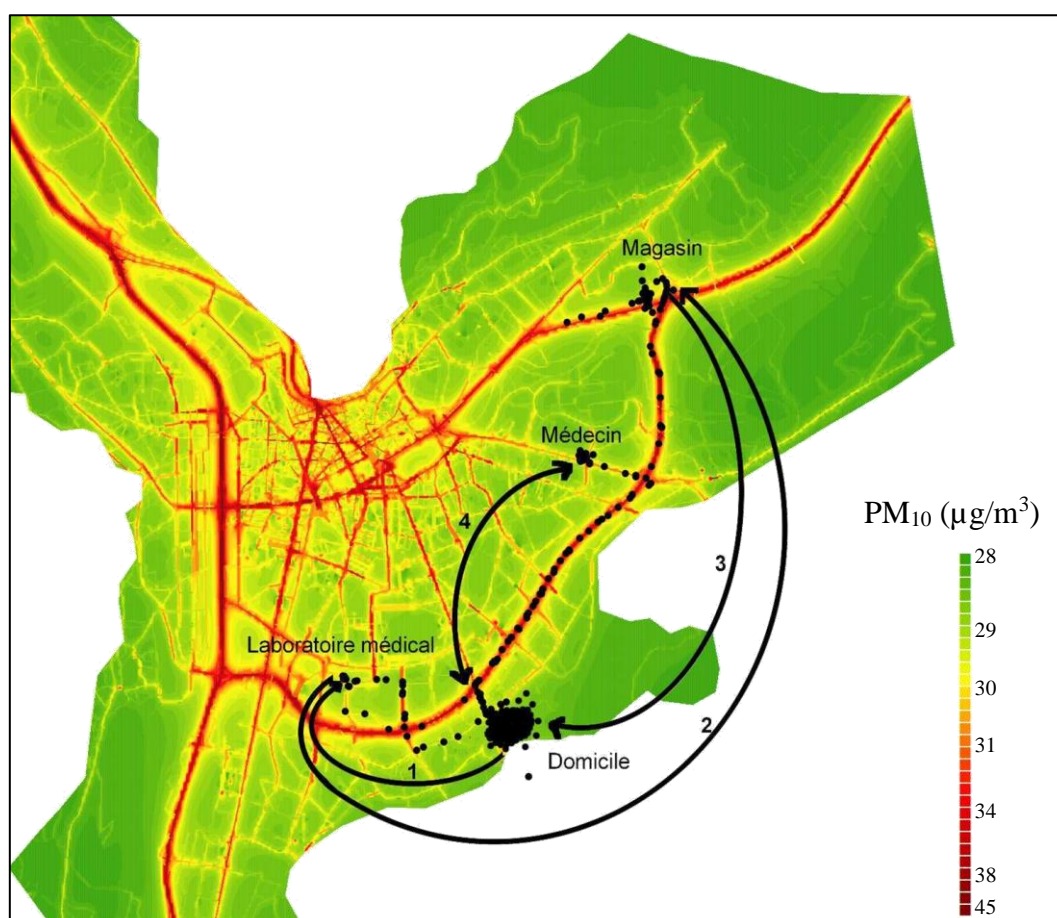
The need to rely on exposure models with higher spatial resolution than monitoring networks has led to the development of atmospheric pollution fine scale models such as dispersion models and land use regression (LUR) models. Dispersion and chemical transport modellings use emission data and dispersion or chemical equations to estimate fine scales map of atmospheric pollution (Valari et al., 2011). Land use regression relies on land use data (traffic and population densities, greenspaces, industries ...), on specific measurements campaigns and on one permanent background air quality monitoring site in order to estimate yearly averages (Beelen et al., 2013; Hoek et al., 2008). Data from air quality monitoring stations can be used to back-extrapolate the yearly average estimated by a LUR model to the subject-specific exposure window of interest (Pedersen et al., 2013a; Slama et al., 2007). Spatial resolution can go from a few meters (see as an example a dispersion model in Figure I-5, next page) for exposure models developed for cities to kilometers for country scale exposure models. In a comparison of the performance of LUR and dispersions models developed for the same area, de Hoogh et al., (2014) observed that the exposure levels at residential home addresses

estimated from exposures models developed in 13 European areas were better correlated for  $\text{NO}_2$  than for  $\text{PM}_{10}$  and  $\text{PM}_{2.5}$ .

*c. Personal monitors*

Personal atmospheric pollutants monitors carried by study subjects allow to take into account all the micro-environments in which subject spends time during the day (indoors, outdoors, during commuting ...). The cost and weight of the samplers -for PM in particular- make them difficult to be carried for a long time and to be used at a large scale. So far, in the context of birth cohorts, very few studies relied on personal monitors (e.g. Slama et al., 2009). As done for example in a feasibility study in Grenoble, it is possible to combine monitoring campaigns at home with a dispersion model and the use of GPS data to take into account space time activity, (Ouidir et al., 2015) (Figure I-5).

**Figure I-5: Example of the use of GPS data with SIRANE dispersion model with a 10x10 m resolution for  $\text{PM}_{10}$  in Grenoble, France**



**Table I-2: Comparison of various outdoor atmospheric pollution exposures assessment used in epidemiological studies**

Model	Input data	Principle	Temporal resolution	Needs to be combined with	Spatial resolution	Pros	Cons	Performance depends on	Particularity
<b>Distance to traffic</b>	<ul style="list-style-type: none"> <li>• Road network</li> </ul>	<ul style="list-style-type: none"> <li>• Distance to the nearest (major) road is used as a proxy</li> </ul>	<ul style="list-style-type: none"> <li>• Annual</li> </ul>	/	/	<ul style="list-style-type: none"> <li>• Easy to use</li> </ul>	<ul style="list-style-type: none"> <li>• Only a proxy</li> <li>• Not temporally adjusted</li> </ul>	<ul style="list-style-type: none"> <li>• Input data</li> </ul>	
<b>Stations</b>	<ul style="list-style-type: none"> <li>• Stations network</li> <li>• Type of station (urban, rural, traffic, industrial ...)</li> </ul>	<ul style="list-style-type: none"> <li>• Use of data from the nearest station</li> </ul>	<ul style="list-style-type: none"> <li>• Daily/hourly</li> </ul>	/	<ul style="list-style-type: none"> <li>• Low, depends of the station network (see Figure I-4)</li> </ul>	<ul style="list-style-type: none"> <li>• Easy to use</li> <li>• Data can be accessed easily</li> <li>• Lots of pollutants</li> <li>• Data available since a long time</li> </ul>	<ul style="list-style-type: none"> <li>• Low spatial resolution</li> <li>• Data cleaning can be troublesome at a country scale</li> <li>• Strong importance of the home-station distance</li> </ul>	<ul style="list-style-type: none"> <li>• Spatial network</li> </ul>	<ul style="list-style-type: none"> <li>• When working on little scale areas, the knowledge of people monitoring the stations is helpful.</li> </ul>
<b>Kriging methods</b>	<ul style="list-style-type: none"> <li>• Measurement campaigns</li> <li>• Stations</li> <li>• Grid</li> </ul>	<ul style="list-style-type: none"> <li>• Estimation of annual exposure by combining measurements and station data</li> <li>• Kriging on a grid using a software</li> <li>• Seasonalization</li> </ul>	<ul style="list-style-type: none"> <li>• Daily/hourly</li> </ul>	/	<ul style="list-style-type: none"> <li>• Depends of the grid used for kriging and of the samplers' location</li> </ul>	<ul style="list-style-type: none"> <li>• No loss of spatial resolution compared to stations</li> </ul>	<ul style="list-style-type: none"> <li>• May smooth extreme value</li> <li>• Software needed for kriging</li> <li>• Cannot capture fine variations due to local sources</li> </ul>	<ul style="list-style-type: none"> <li>• Number of measurements sites</li> <li>• Grid</li> </ul>	
<b>Dispersion, chemical transport and hybrid models</b>	<ul style="list-style-type: none"> <li>• Emissions and sources characteristics inventories</li> <li>• Topography</li> <li>• Equations</li> <li>• Weather</li> <li>• Stations</li> <li>• Grid</li> </ul>	<ul style="list-style-type: none"> <li>• Entry data are used to estimate the pollutants released in the atmosphere</li> <li>• Air pollution is modelled using specific dispersion, transportation and chemical equations</li> <li>• Kriging or estimation on a grid</li> </ul>	<ul style="list-style-type: none"> <li>• If planned or estimated during project: Daily/hourly</li> <li>• If retrospective use of data : annual</li> </ul>	/	<ul style="list-style-type: none"> <li>• Depend of the spatial resolution data (usually high) and the kriging method used (if any)</li> </ul>	<ul style="list-style-type: none"> <li>• Fine spatial and temporal resolutions if fine scale input date</li> </ul>	<ul style="list-style-type: none"> <li>• Need specific training to create the model</li> <li>• Computationally intensive</li> <li>• Input data might be difficult to find</li> <li>• Same than above with seasonalization needed</li> </ul>	<ul style="list-style-type: none"> <li>• Input data</li> <li>• Expertise</li> <li>• Grid</li> <li>• Type of model</li> </ul>	<ul style="list-style-type: none"> <li>• Boundary conditions must be defined</li> <li>• Several software/methods available</li> <li>• Gaussian dispersion models have higher spatial resolution than those estimating average concentration over an area</li> </ul>
<b>Land use regression</b>	<ul style="list-style-type: none"> <li>• Measurement campaign</li> <li>• Station(s)</li> <li>• GIS Predictor</li> <li>• Grid</li> </ul>	<ul style="list-style-type: none"> <li>• Estimation of annual mean at measurements sites</li> <li>• Regression are done with GIS predictors to predict concentrations</li> <li>• Estimation on a grid</li> </ul>	<ul style="list-style-type: none"> <li>• Annual</li> </ul>	• Station	<ul style="list-style-type: none"> <li>• Depends of the land use data resolution, usually high</li> </ul>	<ul style="list-style-type: none"> <li>• Relatively cheap and easy to implement</li> <li>• Easy to estimate at home addresses</li> </ul>	<ul style="list-style-type: none"> <li>• Need training to develop the model</li> <li>• Weather not taken into account</li> </ul>	<ul style="list-style-type: none"> <li>• Input data</li> <li>• Number and characteristics of measurements sites and potential GIS predictors</li> <li>• Grid</li> </ul>	

References: Basagaña et al., 2012; de Hoogh et al., 2014; Lepeule et al., 2010; Sellier et al., 2014; Wang et al., 2015

GIS: Geographic Information System

*d. Estimating exposures during the right time period*

Some exposure models with a high spatial resolution may have a low temporal resolution. For example, LUR models estimate yearly average of exposures. In the case of dispersion models, the method implies to estimate hourly measurements of pollutants in the area considered, but as this represents a huge amount of data, sometimes only the yearly averages are stored and the exposures simulated at a higher temporal resolution are discarded. In environmental epidemiology, however, it is often useful to have exposure levels at subject-specific time periods (e.g. during fetal life) and sometimes back in time (e.g. pregnancy cohort study with recruitment in the 1990's) (Slama et al., 2007). This can be accommodated in two way; the first one is to ask ahead of the project for the daily or hourly data estimated by the dispersion models used for regulatory purposes whenever possible; the second one is to use data from air quality monitoring stations to extrapolate back in time (“back-extrapolate”) and at the appropriate time period (“seasonnalize”). Two different possibilities to seasonnalize and back-extrapolate exposure will be detailed in the method sections, one assuming that spatial exposures in an area varies similarly over time than a reference monitoring station (Methods, III.2.2.b, page 44) (Pedersen et al., 2013a) and the other that the spatial contrasts at the country scale were invariant over time (Methods, II.3.3.b, page 40).



## IV. Human Reproduction

### 1. Overview

Human reproduction is a complex chain of events. The related outcomes expand from intrauterine life (congenital anomalies, fetal growth and rates of miscarriages, stillbirths, and preterm births) to puberty (puberty onset), and to the fecund period (gametogenesis, time to pregnancy, embryo's implantation, menopause onset) until pregnancy (health of the pregnant woman). Studying risk factors of altered fecundity and pregnancy related outcomes is important because the health burden entailed can be large (Slama et al., 2014). This is all the more important in the context of a plausible deterioration of male fecundity parameters in some areas of the world (Auger et al., 1995; Carlsen et al., 1992). Although designs to study pregnancy outcomes such as birthweight are relatively straightforward (cohort of pregnant women, birth registers), studying fecundity is a little more complicated as it implies to identify couples at risk to have a pregnancy and to try including couples remaining infertile.

The outcomes that are considered in this thesis will be described more in details in this chapter.

### 2. Definitions of the main fecundity and pregnancy related outcomes

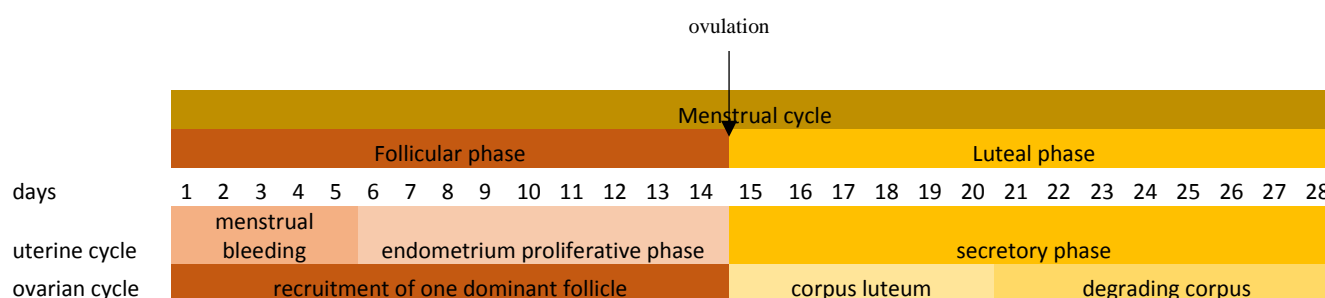
#### *a. Before fertilization*

##### *i. Oogenesis and the menstrual cycle*

Menstrual cycle is the cyclic phenomenon that prepares the female body for pregnancy. It occurs repeatedly (about 450 times for modern women, Thomas and Ellertson, 2000) between puberty and menopause and is controlled by hormonal changes and feedback mechanisms occurring during ovarian and uterine cycles. The first day of the cycle is by convention the first day of the menstrual bleeding (Figure I-6). The ovarian cycle begins by the follicular phase, during which the recruitment of a dominant follicle occurs through multiple follicular waves. Meanwhile, the estradiol secreted by the growing follicle(s) supports the proliferation of the endometrium to prepare the uterus for a possible pregnancy. Peak of estradiol occurs around day 12 and is followed by a rapid increase in luteinizing hormone (LH, delivered by pituitary gland) that triggers ovulation from the dominant follicle around 12 hours later (the pattern in LH surge have variability, Direito et al., 2013). In the second phase -the luteal phase- the follicle that has produced an ovum evolves in corpus luteum under the influence of LH and begins producing progesterone with a peak around 10 days after ovulation. If no fertilization occurs,

the cycle ends once no more progesterone is produced. Once the corpus luteum is in function, the endometrium enters the secretive phase, which is the readiness phase to be implanted by a fertilized egg. When implantation occurs, the implanted embryo will give hormonal signal to the corpus luteum to continue producing progesterone. Otherwise, if no fertilization occurs, once no more progesterone is produced, the upper layer of the endometrium degenerates and is evacuated by the menstrual flow (Jones and Lopez, 2013; Wilcox, 2010).

**Figure I-6: Phases of the menstrual cycle**



The duration of each phase varies by women and within women between cycles. The variability of the cycle length is greater in years following menarche and preceding menopause (Treloar et al., 1967). Most of the variability in cycle length is due to the follicular phase (Fehring et al., 2006). In the French OBSEFF study (Observatory of Fecundity in France, see Methods), the median cycle length was 28 days (5<sup>th</sup>-95<sup>th</sup> percentiles: 23-40, n=127) with a follicular phase lasting in median 18 days (14-27, n=162) and a luteal phase lasting in median 10 days (6-14, n=117) (Rosetta L et al., submitted). In the USA, a study was conducted among 141 women in good health and with regular cycles. This study included 1,060 menstrual cycles and observed a median cycle length of 29 days (95% confidence interval, CI: 22-36), a median follicular length of 16 days (95% CI: 10-22), a median luteal phase of 13 days (95% CI: 9-16) and a median bleeding duration of 6 days (95% CI: 3-8) (Fehring et al., 2006).

Menstrual cycle characteristics are affected by age (Treloar et al., 1967), but can also be associated with ethnicity, smoking status, physical activity, body mass index, alcohol and caffeine consumption, age at menarche, parity, recent oral contraceptive use and marijuana smoking (Jukic et al., 2007; Kato et al., 1999; Liu et al., 2004). Environmental exposures are suspected to influence cycle characteristics, including pesticides (Farr et al., 2004), organochlorine compounds (Cooper et al., 2005; Windham et al., 2005), chlorination by-

products (Windham et al., 2003) and parabens (Nishihama et al., 2016). No study related to atmospheric pollution has to our knowledge been published.

Cycle length can be monitored using diaries filled daily by women, but, as there are no clear external signs for ovulation, knowing follicular and luteal phases length require collecting biological samples and measuring hormonal metabolites (as done for example by Liu et al., 2004; or Windham et al., 2005) or using fecundity monitoring devices such as done by Fehring et al. (2006). Ovulation can also be detected by measuring basal body temperature (as done by Harvey et al., 2009). Studying menstrual characteristics implies recruiting women not using any contraceptive methods or to follow-up women planning to discontinue contraception.

## ii. Spermatogenesis

In males, from puberty onwards, spermatozoa are created continuously in the testis. In humans, spermatogenesis lasts approximately 74 days to obtain spermatozoa from a spermatogonium (Amann, 2008; Heller and Clermont, 1964). The duration of approximately 64 days is sometimes found, but this estimate ignores the 10 days needed by the proliferation of spermatogonia (Amann, 2008).

Factors influencing spermatogenesis and semen parameters include age (Stone et al., 2013), lifestyle factors (Jensen et al., 2004; Taha et al., 2012) but also occupational exposures (De Fleurian et al., 2009).

### b. *Between fertilization and pregnancy detection*

#### i. Fecundity, fertility, fecundability and time to pregnancy

*The following definitions will be used through all the thesis.*

**Fecundity** is the biological aptitude to conceive and bear a child until birth. This biological parameter cannot be assessed directly and can either be defined for a person or a couple (Leridon, 2007).

**Fecundability** is the monthly probability to conceive for a couple not using any contraceptive method (Gini, 1926; Slama et al., 2013).

**Fertility** is a demographic parameter measuring the number of children per woman (Leridon, 2007).

**Time to pregnancy** is the time needed by a woman or a couple not using contraception to obtain a pregnancy ending by a live birth (Baird et al., 1986).

**Involuntary infertility** is usually defined as a period of unprotected intercourse without succeeding in having a child; one can for example consider 12 or 24 months involuntary infertility. (Habbema et al., 2004; Slama et al., 2012)

As identifying women who have already been pregnant can be done without too much difficulties, the classical design to study fecundity is the pregnancy-based design in which women were asked to provide retrospectively their duration of unprotected intercourse before being pregnant (as done for example in the French PELAGIE cohort by Chevrier et al., 2013 or in the Danish National Birth Cohort by Bach et al., 2015). Fecundability can be assessed by estimating the percentage of women conceiving during the first month of unprotected intercourse (as done in a birth cohort by Slama et al., 2013). The major issue with this retrospective design is that couples never achieving pregnancy are not included (Slama et al., 2014). To include these couples, recruitment should take place *before* the start of the period of unprotected intercourse (as done for example in LIFE study, Buck Louis et al., 2011; or in Germany for couples using natural family planning, Gnoth et al., 2003) or *during* this period (as in the French OBSEFF study by Slama et al., 2012 or in the Danish *Snart gravid* study by Mikkelsen et al., 2009).

Statistical methods and potential biases depend on when the couples were recruited: before the period of unprotected intercourse (incident cohort), during (current duration study if couples are not followed-up, prevalent cohort design otherwise) or after (pregnancy-based). Study designs and biases in time to pregnancy studies have been summarized elsewhere (Slama et al., 2006, 2014, Weinberg et al., 1993, 1994) and will be further discussed here in the discussion (Chapter IV, III.4, page 161).

Fecundity and fecundability depend on male and on female factors that can impact sperm or egg quality or viability of the embryo, such as age (Mutsaerts et al., 2012), active smoking (Baird and Wilcox, 1985), body mass index (Gesink Law et al., 2007), lifestyle (Curtis et al., 1997; Hassan and Killick, 2004), medical treatment and health (female asthma for example is associated with prolonged time to pregnancy, Juul Gade et al., 2014).

ii. *Pregnancy detection*

Pregnancy can be detected by the woman noticing a delay in her menstrual periods. It can also be detected earlier by measures in blood or urine of Human Chorionic Gonadotropin (hCG), a hormone secreted after implantation.

c. *After fertilization*

i. *Gestational age at birth, preterm and very preterm delivery*

Gestational age at birth is the time between the beginning of the last menstrual period and birth; it corresponds approximately to the time between fertilization and birth, with two weeks being added to account for the duration of the follicular phase and for the time between ovulation and fertilization (Figure I-7, page 21). The gestational age estimated from the last menstrual period can be corrected at the first ultrasound measurement if the size of the embryo is smaller or bigger than expected at this age from reference charts (Gjessing et al., 2007). Although ultrasound measurements give a good estimate of the delivery date, in term of epidemiology, it can be debated to rely on it because if an exposure has an effect on the early embryo development, correcting the gestational age might introduce bias in the association to be identified (Basso and Olsen, 2007; Lynch and Zhang, 2007).

Preterm delivery corresponds to births occurring before 37 completed weeks of gestation while very preterm delivery is usually defined as births occurring before 32 completed weeks of gestation (Figure I-7). As the newborn is still immature after 37 completed weeks of gestation, there is an increased risk of morbidity and mortality for newborns born before term (Goldenberg et al., 2008). Preterm births might be due to malformations (Purisch et al., 2008), maternal pathologies such as infections (Rours et al., 2011) or pre-eclampsia (hypertensive diseases of unknown causes occurring in 2-8% of pregnancies, Ananth et al., 2013; English et al., 2015; Wallis et al., 2008, with risk factors including obesity, age and multiple pregnancy; Duckitt and Harrington, 2005; Sibai et al., 2005). Other factors such as maternal smoking, maternal obesity and maternal obstetric history (parity, previous history of preterm birth) and gender of the fetus are also associated with increased preterm delivery risk (reviewed by Os et al., 2013).

## ii. Pregnancy losses, miscarriages and stillbirths

Pregnancy losses occurring before the pregnancy detection are called early pregnancy loss, while those occurring once the fetus is viable are called stillbirth (opposed to live birth, which is when the child is born alive, even if he decease shortly after birth) (Wilcox, 2010). The legal limit between miscarriage and stillbirth is different by country<sup>8</sup>. In France, to be considered viable, a child must be born with a gestational age of at least 22 weeks with a birthweight of at least 500g.

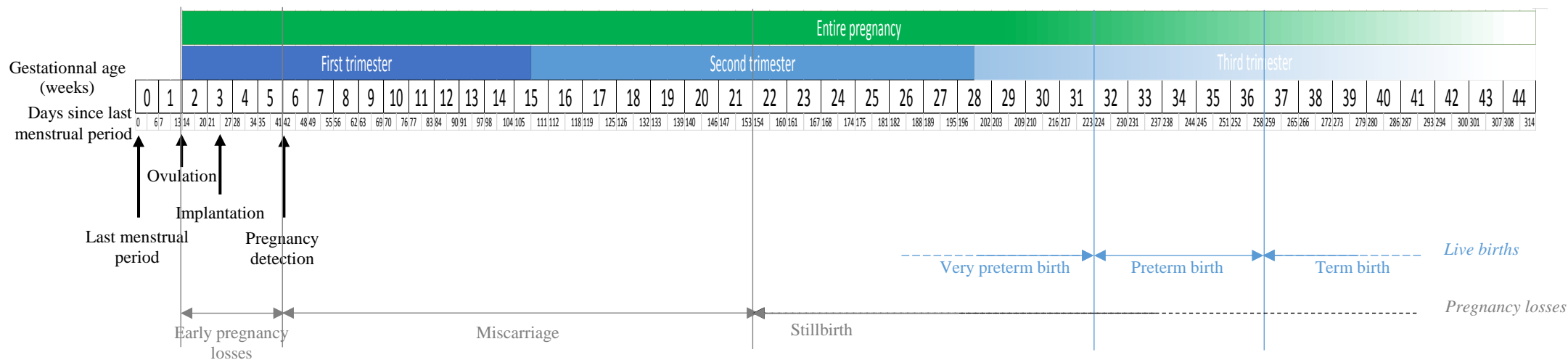
Pregnancy losses occurring between fertilization and pregnancy detection might be unnoticed. This might concern a large number of pregnancies; in a study conducted between 1982 and 1985, 25% of the pregnancies ended within 6 weeks after LMP (Wilcox et al., 1990).

Risk factors for stillbirth include prolonged pregnancy, congenital abnormalities, old maternal age, maternal infections and lifestyle factors (Lawn et al., 2016). For miscarriage, risk factors include chromosomal aberrations, maternal obstetric history, infections, uterine malformations, lifestyle factors, and age (García-Enguíanos et al., 2002).

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<sup>8</sup> Etude de législation comparée n°184, « Les enfants nés sans vie », (Sénat, 2008)

**Figure I-7: gestational age, preterm delivery and pregnancy losses**



sources : (Slama et al., 2014; Wilcox, 2010)

### 3. Human reproduction and atmospheric pollution

Several studies were conducted on the relation between human reproduction and atmospheric pollution, with some aspects more studied than others. In this section, the studies describing a possible effect of atmospheric pollution on human reproduction are reviewed.

#### *a. Air pollution effect on gametogenesis*

On the female side, to our knowledge, no study has been conducted on the possible influence of atmospheric pollution on menstrual function in Human yet.

Studies on sperm quality have been recently reviewed by Deng et al. (2016), Fathi Najafi et al. (2015) and Lafuente et al. (2016). The first review included the results from 10 studies and performed a meta-analysis concluding that there was a trend for impaired sperm quality in the most exposed group compared to the lowest exposure group (Deng et al., 2016). The second review included 17 studies and concluded, without a meta-analysis, that air pollution influence DNA fragmentation and sperm morphology but not sperm motility (Lafuente et al., 2016). A previous review and meta-analysis focused on slightly different publications than Deng et al. (2016) concluded on the contrary that air pollution was associated with decreased sperm motility but that there was no evidence regarding the other parameters (Fathi Najafi et al., 2015). The fact that the reviews did not share the same conclusion is probably due to the difficulty that reviews had to compare the various existing studies on this topic, in particular due to the lack of standardized measurements of sperm parameters.

#### *b. Air pollution effect on fecundity, fertility, and fecundability*

The studies conducted on fecundity related outcomes have been recently reviewed (Checa Vizcaíno et al., 2016; Frutos et al., 2015) and are summarized by outcome in Table I-3, restricting to studies in the general population. In the general population, four studies were conducted: one on fecundability (Slama et al., 2013), two on fertility rates (Koshal et al., 1980; Nieuwenhuijsen et al., 2014) and one on incident involuntary infertility (Mahalingaiah et al., 2016). Few studies were also conducted on specific populations, generally on couples resorting to In Vitro Fertilization (IVF) or Intracytoplasmic Sperm Injection (ICSI) (Legro et al., 2010; Perin et al., 2010a, 2010b). Most of the studies on fecundity related outcomes reported that atmospheric pollution was associated with altered reproductive outcomes. No study was conducted on time to pregnancy in a general population and the three studies focusing on couples with difficulties to conceive were not able to adjust for possible cofounders except age



or season. The study on fecundability observed reduced fecundability with increased exposure to NO<sub>2</sub> and PM<sub>2.5</sub> around contraception stop (Slama et al., 2013) while the study on incident infertility observed a small increase in risk of involuntary infertility when distance to traffic was used to assess exposure, but not when it was assessed using a dispersion model (Mahalingaiah et al., 2016). With the design of this last study, it was not possible to consider short-term effects of air pollutants as the exact diagnosis date was unknown and set as the average between the dates of two questionnaires separated by 2 years.

**Table I-3: Publications on air pollution and fecundity-related outcomes in Human.**

Outcome	Study	Location	Period	Population	Sample size	Exposure windows	Exposure considered, (distribution) and exposure model	Adjustment factors	Statistical model	Main findings (95% CI)
Fecundability	Slama et al., 2013	Teplice, Czech Republic	1994-1996	Birth cohort: pregnancy-based fecundability design. Planned pregnancies only	1,916 (25% obtained pregnancy in the first month of unprotected intercourse)	lag1 :30 days after contraception stop lag2: 30 days before contraception stop [lag1 lag2]: from 30 days before contraception stop to 30 days after	(25 <sup>th</sup> , 50 <sup>th</sup> , 75 <sup>th</sup> percentiles in lag1, µg/m <sup>3</sup> ) PM <sub>2.5</sub> , (27, 34, 43) O <sub>3</sub> , NO <sub>2</sub> , (31, 36, 40) SO <sub>2</sub> Model= one station for the whole study area, <12 km from home	Maternal smoking, body mass index, maternal age at the start of the period of unprotected intercourse, education, marital status, parity, respiratory epidemic in the previous month, time of the start of the period of unprotected intercourse (spline)	Binomial regression model with a logarithmic link	Fecundability ratio per each increase by 10µg/m <sup>3</sup> in exposure: [lag1] PM <sub>2.5</sub> : 0.96 (0.86;1.07) O <sub>3</sub> 1.06 (0.97;1.15) NO <sub>2</sub> <b>0.71 (0.57;0.87)</b> SO <sub>2</sub> 0.99 (0.94;1.05) [lag1 lag2] PM <sub>2.5</sub> : <b>0.78 (0.65;0.94)</b> O <sub>3</sub> 1.04 (0.93;1.17) NO <sub>2</sub> <b>0.72 (0.53;0.97)</b> SO <sub>2</sub> 0.94 (0.85;1.04)
Incident involuntary infertility (attempted conception ≥12 months without success).	Mahalingaiah et al., 2016	USA	1993-2003	Prospective cohort (Nurses' Health Study II). Questionnaires every 2 years	36,294 (2,508 cases for 213,416 person-years)	2 years prior diagnosis 4 year prior diagnosis Cumulative: 1998 to current	(Median and IQR during the 2 years average, µg/m <sup>3</sup> ) PM <sub>10</sub> (24, 7) PM <sub>2.5</sub> (15, 4) PM <sub>coarse</sub> (9, 5) Nationwide spatiotemporal model, Exposure at home address	Age, race, calendar year, region, current body mass index, smoking, oral contraceptive use, age at menarche, overall diet quality, history of rotating shift work and census tract level median income and home value	Time-varying Cox proportional hazard model	Hazard ratio per 10 µg/m <sup>3</sup> increase: PM <sub>10</sub> 2 years 1.04 (0.96;1.11) 4 years 0.99 (0.91;1.08) Cumulative 1.06 (0.99;1.13) PM <sub>2.5</sub> 2 years 0.98 (0.86;1.12) 4 years 0.91 (0.78;1.05) Cumulative 1.05 (0.93;1.20) PM <sub>coarse</sub> 2 years 1.10 (0.981.23) 4 years 1.05 (0.93;1.19) Cumulative 1.10 (0.99;1.22)
Fertility rates (number of children ever born per 1000 ever married women in a given age group)	Koshal et al., 1980	USA, 74 standard metropolitan statistical areas	1970			annual	PM per standard metropolitan statistical area	Income, percentage of families below the low income level, annual temperature, education of females relative to male, availability of medical facilities and rate of net migration	Multivariate linear regressions	Air pollution reduced fertility rates.
Fertility rates (number of live births per 1000 women ages between 15 and 44 by census tract)	Nieuwenhuijsen et al., 2014	Barcelona, Spain	2011-2012	Cross sectional study, registries	27,617 births	year 2009	(IQR, µg/m <sup>3</sup> by default) PM <sub>10</sub> (2.9) PM <sub>2.5</sub> (2.5) PM <sub>coarse</sub> (3.5) PM <sub>2.5</sub> absorbance (0.7 unit) NO <sub>2</sub> (12.0) NO <sub>x</sub> (26.0) LUR (ESCAPE), exposure averaged by census tract area	Socioeconomic status, age, percentage of women born outside Spain.	Besag-York-Mollié models	Infertility risk per IQR increase in air pollution: NO <sub>2</sub> 0.97 (0.94;1.00) NO <sub>x</sub> 0.99 (0.96;1.02) PM <sub>2.5</sub> 0.99 (0.96;1.02) PM <sub>10</sub> 0.99 (0.97;1.02) PM <sub>coarse</sub> <b>0.88 (0.83;0.94)</b> PM <sub>2.5</sub> 0.98 (0.95;1.02)

NO<sub>2</sub>: nitrogen dioxide; NO<sub>x</sub>: nitrogen oxides; O<sub>3</sub>: ozone; PM: particulate matter; SO<sub>2</sub>: sulfur dioxide;  
IQR: Interquartile Range

*c. Air pollution effect on birth weight, preterm delivery and stillbirth*

Many studies and reviews have been conducted on birth weight and preterm delivery. Not exhaustively, association of air pollution with (low) birthweight and preterm birth were reviewed by at least eight reviews (Bonzini et al., 2010; Shah et al., 2011; Srám et al., 2005; Stieb et al., 2012), with some reviews focusing only (Bosetti et al., 2010; Lamichhane et al., 2015) or mostly (Backes et al., 2013) on PM and birth outcomes or PM and preterm birth (Sun et al., 2015). The reviews concluded that despite significant heterogeneity between studies, PM (Lamichhane et al., 2015; Shah et al., 2011; Stieb et al., 2012; Sun et al., 2015) and other pollutants (Shah et al., 2011; Stieb et al., 2012) might increase preterm delivery risk. Srám et al. (2005) concluded that it was possible that air pollution increase preterm delivery risk while Bosetti et al. (2010) did not conclude in favor of such an association. Articles included in the review done by Sun *and colleagues* (2015), the most recent review on the association between PM<sub>2.5</sub> and preterm birth risk, are summarized in Table I-4, page 27.

Reviews by Backes et al. (2013), Bonzini et al. (2010), Lamichhane et al. (2015), Shah et al. (2011), Srám et al. (2005) and Stieb et al. (2012) were in favor of an association between air pollution and birthweight while Bosetti et al. (2010) did not conclude for an association. A risk-assessment study based on the risk of term low birth weight due to exposure to PM<sub>2.5</sub> reported by Pedersen et al. (2013a) estimated that 24% of total cases (9-37%) in the French urban area of Grenoble and 28% of cases (11-43%) in the bigger French urban area of Lyon were attributable to exposure to PM<sub>2.5</sub> (Morelli et al., 2016).

Risk of stillbirth was recently reviewed by Siddika et al. (2016). A meta-analysis was performed on the 13 epidemiological studies identified and the authors concluded were that their results provided suggestive evidence that air pollution is a risk factor for stillbirth.

#### 4. Human reproduction and meteorological parameters

Meteorological parameters influence both atmospheric pollution levels and human health (see McMichael et al., 2006, for a review in the context of climate change). Studies sometimes consider meteorological parameters -especially temperature- as confounding factors when studying association between atmospheric pollutants and human reproductive health (e.g. preterm birth: Chang et al., 2015; stillbirth: Faiz et al., 2012; sperm characteristics: Sokol et al., 2006), but most studies adjusted only for season. Some studies focused on the effect of temperature on birth outcomes (reviewed by Strand et al., 2011), climate and birth outcomes (Beltran et al., 2014; Poursafa et al., 2015) and temperature and preterm delivery (Carolan-Olah and Frankowska, 2014). Regarding fecundity, no strong reasons of considering meteorological factors on time to pregnancy or menstrual cycle were found.

**Table I-4: Characteristics of the studies on preterm delivery and PM<sub>2.5</sub> exposure reviewed by Sun et al. (2015)**

Study	Population (n, % preterm) Location and period	Exposure (air pollution and meteorology)	Statistical model and exposure windows	Main findings (95% CI) regarding preterm birth risk. For results given by IQR increase, the value in parenthesis after the exposure windows corresponds to IQR
Brauer et al., 2008	Registers (n=70,249, 5%) Vancouver, Canada, 1999-2002	CO, NO, NO <sub>2</sub> , O <sub>3</sub> , PM <sub>10</sub> , PM <sub>2.5</sub> , SO <sub>2</sub> , black carbon Nearest station, LUR, inverse- distance weighting	Logistic regression WP, M1, M1r, M3r	“For the preterm birth outcome of < 37 weeks, we did not observe any consistent associations with any of the pregnancy average exposure metrics except for PM <sub>2.5</sub> (inverse distance weighting: OR= 1.06; 95% CI, 1.01–1.11).”
Chang et al., 2015	Register (Atlanta Birth Cohort) (n=175,891, 10.6%) Atlanta, USA 1999-2005	PM <sub>2.5</sub> monitoring stations Temperature Monitoring stations	Distributed exposure discrete-time survival model WP, T1, T2, T3, M1 4-week lag (week t-3 to t)	Relative risk per one IQR increase in PM <sub>2.5</sub> WP (3.1 µg/m <sup>3</sup> ) 1.03 (1.00, 1.06) T3 (5.4 µg/m <sup>3</sup> ) 1.00 (0.99, 1.01) T1 (5.2 µg/m <sup>3</sup> ) 1.01 (0.99, 1.04) 1rst 4w (5.9 µg/m <sup>3</sup> ) 1.00 (0.99, 1.02) T2 (5.1 µg/m <sup>3</sup> ) 1.03 (1.01, 1.05) 4 week lag (6.0 µg/m <sup>3</sup> ) 1.00 (0.99, 1.02)
Fleischer et al., 2014	Cross-sectional survey (n=192,900, 3-11%) 24 countries (Africa, Latin America, Asia) 2004-2008	PM <sub>2.5</sub> Satellites combined with chemical transport model and monitoring stations, buffer of 50 km around each health facilities	Generalized estimating equation models (nested structure) with logit link T1, last month	Odds ratio for an increase by 10 µg/m <sup>3</sup> of PM <sub>2.5</sub> in month before birth Whole survey : 0.96 (0.90, 1.02) China : 1.11 (1.04, 1.17) India : 0.96 (0.91, 1.03)
Gehring et al., 2011a	Cohort (PIAMA) (n=3,863, 4%) Netherlands 1996-1997	NO <sub>2</sub> , PM <sub>2.5</sub> , soot LUR	Logistic regression WP, T1, last month	Odds ratio per one IQR increase NO <sub>2</sub> (IQR in µg/m <sup>3</sup> ) PM <sub>2.5</sub> (IQR in µg/m <sup>3</sup> ) Soot (IQR in 10 <sup>-5</sup> m <sup>-1</sup> ) WP (11) 1.08 (0.80;1.47) WP (5) 1.22 (0.83; 1.80) WP (1) 1.27 (0.96; 1.67) T1 (14) 0.97 (0.73; 1.27) T1 (8) 0.98 (0.75; 1.29) T1 (2) 0.94 (0.72; 1.23) LM (14) 1.08 (0.86;1.36) LM (5) 1.06 (0.84;1.35) LM (1) 1.12 (0.96;1.32)
Gray et al., 2014	Register (n=457,642, 10%) North Carolina, USA 2002-2006	PM <sub>2.5</sub> , O <sub>3</sub> Station and numerical models	Logistic mixed regression WP	Per one IQR increase: PM <sub>2.5</sub> : WP (14 µg/m <sup>3</sup> ) 1.01 (0.99, 1.02) O <sub>3</sub> : WP (6 ppb) 1.02 (0.99, 1.04)
Ha et al., 2014	Register (n=423,719, 9%) Florida, USA 2004-2005	PM <sub>2.5</sub> , O <sub>3</sub> Hierarchical bayesian prediction model	Logistic regression WP, T1, T2, T3	Odds ratio per one IQR increase : O <sub>3</sub> (IQR in ppb) PM <sub>2.5</sub> (IQR in µg/m <sup>3</sup> ) WP (7) 1.03(1.01,1.05) WP (2) 1.05(1.04,1.07) T1 (8) 1.01 (1.0,1.03) T1 (3) 1.03 (1.02, 1.04) T2 (8) 1.02(1.01,1.04) T2 (3) 1.12 (1.11, 1.14) T3 (8) 0.99 (0.98,1.01) T3 (3) 1.03 (1.01, 1.04)
Hannam et al., 2014	Cohort (North West England Perinatal Survey) (n=252,170, 7%) England, UK 2004-2008	NO <sub>2</sub> , NO <sub>x</sub> , PM <sub>10</sub> , PM <sub>2.5</sub> , CO Seasonalized (monthly) spatial model (1x1 km) Nearest station	Logistic regression WP, T0, T1, T2, T3	Odds ratio per one IQR increase, Spatio-temporal model NO <sub>2</sub> : WP 1.10 0.82–1.48 WP 1.03 0.93–1.14 T1 1.08 0.92–1.27 T1 1.04 0.91–1.19 T2 1.07 0.91–1.25 T2 1.08 0.94–1.24 T3 1.01 0.86–1.19 T3 1.00 0.87–1.15 PM <sub>10</sub> : WP 0.98 0.85–1.12 WP 0.90 0.74–1.11 T1 1.06 0.92–1.21 T1 1.00 0.90–1.12 T2 0.95 0.83–1.09 T2 0.98 0.92–1.05 T3 0.97 0.84–1.11 T3 0.91 0.82–1.02
Huynh et al., 2006	Case-control (Register) (n=42,692) California, USA 1998-2000	CO (ppm), PM <sub>2.5</sub> Nearest station	Conditional logistic regression (matching) WP, M1, last 2 week	OR, PM <sub>2.5</sub> , per 10 µg/m <sup>3</sup> increase WP <b>1.15 [1.15, 1.16]</b> First month <b>1.13 [1.13, 1.13]</b> Last 2 weeks <b>1.06 [1.05, 1.06]</b> PM <sub>2.5</sub> estimates not modified with adjustment for CO

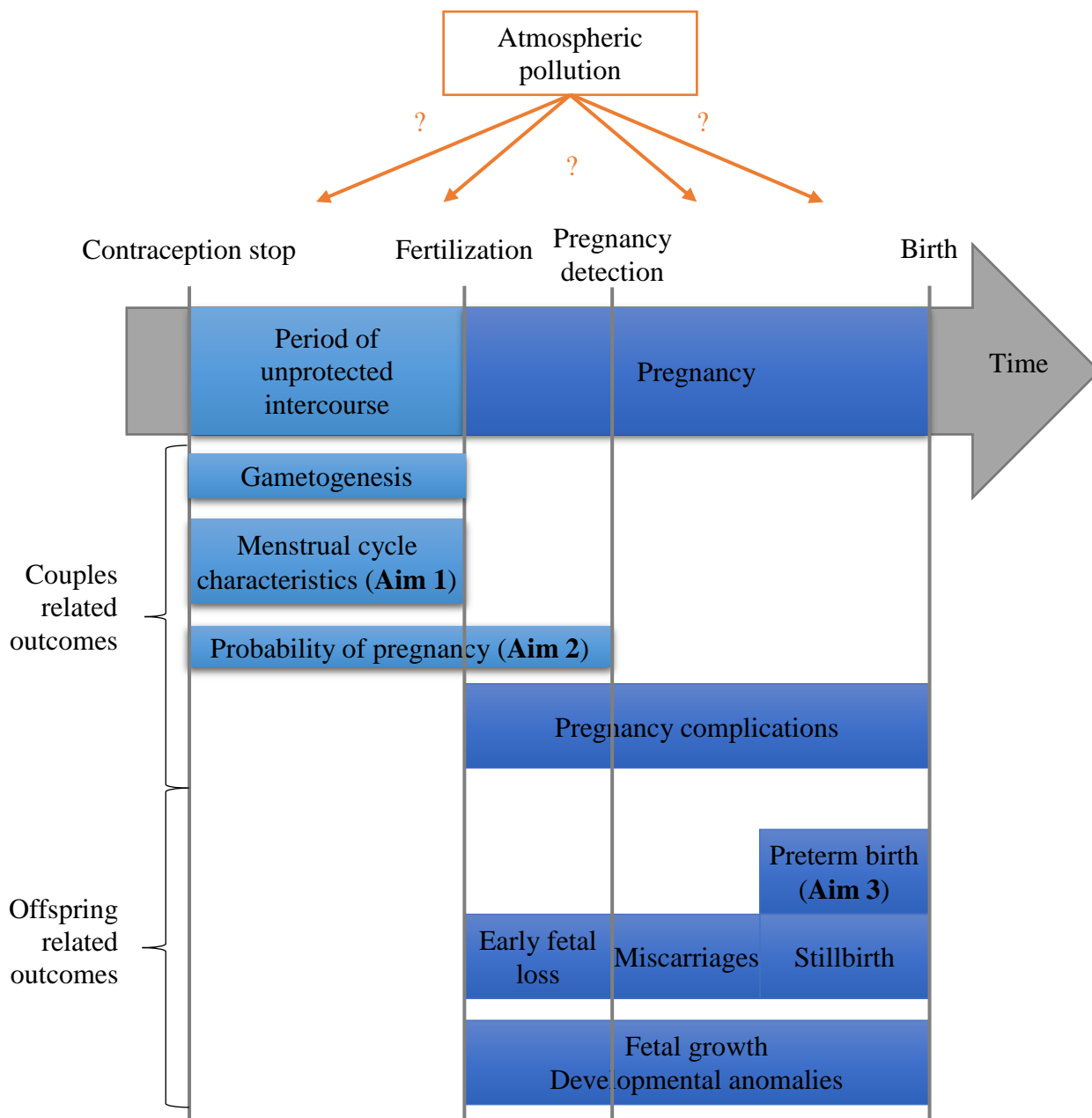
Study	Population (n, % preterm) Location and period	Exposure (air pollution and meteorology)	Statistical model and exposure windows	Main findings (95% CI) regarding preterm birth risk. For results given by IQR increase, the value in parenthesis after the exposure windows corresponds to IQR
Hyder et al., 2014	Register (662,921, 6%) Connecticut and Massachusetts, USA 2000-2006	PM <sub>2.5</sub> Monitors, satellite	Logistic regression Model for trimesters included residuals from regressing exposure estimates from the trimester of interest against other trimesters. WP, T1, T2, T3	Air pollution estimated using monitors, Odds ratio per 2.4 µg/m <sup>3</sup> increase (one IQR) WP 1.00 (0.99 to 1.02) T1 1.00 (0.99 to 1.02) T2 1.01 (1.00 to 1.02) T3 1.00 (0.99 to 1.01) Air pollution estimated using satellites model: no association
Jalaludin et al., 2007	Register (Midwives Data Collection) (n=123,840, 5%) Sydney, Australia 1998-2000	CO, NO <sub>2</sub> , O <sub>3</sub> , PM <sub>10</sub> , PM <sub>2.5</sub> , SO <sub>2</sub> Average of all station or matching by postcode Relative humidity and temperature (tested with linear coding only)	Logistic regression T1, T3r, M1, M1r	Odds ratio by 1 unit increase: PM <sub>10</sub> (µg/m <sup>3</sup> ) M1r 0.99 (0.98–1.00) T3r 0.99 (0.98–1.00) <b>M1 0.98 (0.97–0.99)</b> T1 0.99 (0.97–1.00) PM <sub>2.5</sub> (µg/m <sup>3</sup> ) M1r 0.98 (0.96–1.01) T3r 0.98 (0.95–1.01) M1 0.98 (0.96–1.00) T1 0.98 (0.95–1.01) NO <sub>2</sub> (pb) M1r 1.00 (0.99–1.01) T3r 1.01 (0.99–1.02) <b>M1 0.97 (0.96–0.98)</b> <b>T1 0.97 (0.96–0.98)</b>
Kloog et al., 2012	Registry (Massachusetts birth registry) (n=634,244, 10 % ?) Massachusetts, USA 2000-2008	PM <sub>2.5</sub> satellites	Logistic mixed regression models using pre term/full term birth as the outcome (random intercept for census tract) WP, T3r, M1r	Odds ratio for each 10 µg/m <sup>3</sup> increment M1r 1.00 (0.96;1.04) T3r 0.99 (0.94;1.03) <b>WP 1.06 (1.01;1.13)</b>
Lee et al., 2013	Hospital-based cohort (n=34,705, 9%) Pittsburgh, USA 1997-2002	PM <sub>10</sub> , PM <sub>2.5</sub> (µg/m <sup>3</sup> ), O <sub>3</sub> (ppb) kriging method, zip code	Logistic regression T1	Odds ratio by one IQR increase during first trimester: PM <sub>10</sub> (8 µg/m <sup>3</sup> ) 1.04 (0.94–1.14) PM <sub>2.5</sub> (4 µg/m <sup>3</sup> ) <b>1.10 (1.01–1.20)</b> O <sub>3</sub> (17 ppb) <b>1.23 (1.01–1.50)</b>
Pereira et al., 2014a	Birth registry 29,175 women giving birth to 61,688 neonates (6% in reference population) Connecticut, USA 2000-2006	PM <sub>2.5</sub> , CO, NO <sub>2</sub> Station  Temperature station	Conditional logistic regression, pregnancies matched by mother WP, T1, T2, T3 WP and T3 are censored	Odds ratio per one IQR increase for PM <sub>2.5</sub> (Results with other pollutants not shown) <b>WP (2 µg/m<sup>3</sup>) 1.13 (1.00, 1.28)</b> <b>T1 (3 µg/m<sup>3</sup>) 1.10 (1.03, 1.17)</b> <b>T2 (3 µg/m<sup>3</sup>) 0.93 (0.87, 0.99)</b> <b>T3 (4 µg/m<sup>3</sup>) 1.06 (1.00, 1.11)</b>
Pereira et al., 2014b	Case-control, Registry 31,567 births for 14,497 women (7% in reference population) Perth, Australia. 1997-2007	PM <sub>2.5</sub> , CO, NO <sub>2</sub> , O <sub>3</sub> station	Conditional logistic regression, pregnancies matched by mother (same as design as Pereira et al., 2014a) WP, T1, T2, T3 WP and T3 are censored	Odds ratio per 1 µg/m <sup>3</sup> increase in PM <sub>2.5</sub> WP 0.99 (0.95, 1.04) T1 1.00 (0.96, 1.04) T2 1.00 (0.96, 1.04) T3 0.98 (0.94, 1.02) Association with other pollutants not reported
Ritz et al., 2007	Case-control nested in birth cohort (n=58,316) California, USA 2003	CO, NO <sub>2</sub> , O <sub>3</sub> , PM <sub>2.5</sub> Station ZIP codes	Logistic regression WP, T1, 6 weeks before birth	“exposure to the traffic-related pollutants -CO and fine particles- mostly during the first trimester but also possibly high exposures prior to delivery are associated with preterm birth in the Los Angeles metropolitan area. Importantly, the results were not confounded by well-known risk factors missing from California birth certificates.”
Rudra et al., 2011	Cohort (Omega study) (n=3,509, 11%) Washington, USA 1996-2006	CO, PM <sub>2.5</sub> Regression model (take temperature in account)	Logistic regression T1, T2, T3r	Odds ratio: CO (by 0.1ppm increase) PM <sub>2.5</sub> (by 0.5 µg/m <sup>3</sup> increase) Last 90 days 0.98 (0.94–1.01) Last 90 days 0.99 (0.96–1.02) Results for other exposure windows not shown.
Wilhelm and Ritz, 2005	Registry 106,483 (9%) California, USA 1994-2000	CO, NO <sub>2</sub> , O <sub>3</sub> , PM <sub>10</sub> , PM <sub>2.5</sub> Nearest station	Logistic regression T1, T2, M1, 6 weeks before birth	Relative risk for ZIP code PM <sub>10</sub> (per 10 µg/m <sup>3</sup> ) T1 0.99 (0.96–1.01) Last 6 weeks 1.02 (0.99–1.04) PM <sub>2.5</sub> (per 10 µg/m <sup>3</sup> ) T1 <b>0.73 (0.67–0.80)</b> Last 6 weeks <b>1.10 (1.00–1.21)</b>
Wu et al., 2009	Hospitals databases (n=81,186, 8%) Los Angeles, USA 1997-2006	NO <sub>x</sub> , PM <sub>2.5</sub> dispersion	Logistic regression WP	Odds ratio per one IQR increase : NO <sub>x</sub> (6 ppb) 1.06 (1.03–1.09) PM <sub>2.5</sub> (1 µg/m <sup>3</sup> ) 1.03 (1.01–1.06)

WP : whole pregnancy, T1, T2, T3: first , second, third trimesters of pregnancy, M1: first month of pregnancy, M1r: last month of pregnancy, T3r: last 90 days of pregnancy. ppb: parts per billion; IQR: interquartile range

## V. Objectives of the thesis

The aim of this thesis is to quantify the association between atmospheric pollution and specific health outcomes related to human reproduction. First, menstrual cycle data will be studied (Aim 1). Then two study designs on the same population will allow to study a marker of fecundity (Aim 2). Finally, preterm birth will be studied using pooled data from twelve European birth cohorts (Aim 3) (Figure I-8).

**Figure I-8: overview of the timeline of reproduction-related outcomes (adapted from Slama, 2014)**









## Chapter II: Methods



## I. French Summary

Ce chapitre donne des précisions sur les méthodes utilisées et les populations étudiées. Elle peut être ignorée en première lecture car les méthodes sont précisées dans chaque article. Le doctorat se base sur deux études, l'observatoire de la fertilité en France et le consortium ESCAPE.

L'OBSERVatoire de la Fertilité en France (OBSEFF) est constitué d'un échantillon aléatoire de couples de la population générale dont la femme était âgée de 18 à 44 ans au recrutement en 2007. Les 943 couples n'utilisant pas de méthode contraceptive recrutés en 2007 et un échantillon aléatoire de 817 couples sous contraception ont été suivis pendant deux ans en répondant à un questionnaire annuel sur leur santé reproductive. Les femmes n'utilisant pas de méthode contraceptive pouvaient, si elles le souhaitaient, participer à une étude supplémentaire sur le cycle menstruel en remplissant un carnet et en recueillant la première urine de la journée pendant un cycle. Cela a permis de déterminer la date d'ovulation à partir de dosages d'un métabolite de la progestérone et de définir plusieurs caractéristiques du cycle menstruel de 184 participantes.

L'European Study of Cohorts for Air Pollution Effects (ESCAPE) est une étude européenne regroupant plusieurs cohortes pour lesquelles les expositions à la pollution atmosphérique ont été estimées de manière standardisées à l'aide de modèles Land Use Regression (LUR). Les données de santé transférées par les treize cohortes (n=71 493) participant à l'étude sur la prématurité ont été harmonisées et poolées tandis que les estimations à la pollution atmosphérique ont été saisonnalisées et back-extrapolées en prenant si possible les déménagements en compte.

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*This chapter can be skipped as its purpose is to give more details than the method sections of the articles provided in the following chapters.*

## II. OBSEFF study

### 1. Population

OBSEFF study (Observatoire Epidémiologique de la Fertilité en France) was conducted in France in 2007-2009. It aimed to describe fecundity and menstrual cycle of women from the general population and to study the potential influence of atmospheric pollutants on those parameters (Slama et al., 2012).

A random sampling of couples from the general population was recruited by a phone survey in 2007. Random landline phone numbers over-sampling urban areas with more than 100,000 inhabitants were generated, allowing to contact 64,262 homes. When a woman aged 18-44 was living in the contacted home, she was asked to answer a short eligibility questionnaire, while if more than one woman can be included, the questionnaire was asked to the woman who will have her birthday the sooner. A total of 19,121 women answered the eligibility questionnaire. Women aged 18 to 44, living or having a regular relationship with a male partner and not using regularly any contraceptive method were included provided they have had sexual intercourse during the last month and not delivered during the last three months. Eligible women (n=943) answered a long questionnaire (named Q0) and were asked to participate in an ancillary study with hormonal assessment of menstrual cycle. 678 accepted to participate and received ethical agreement forms, a booklet calendar and pH strips. During one menstrual cycle, each woman had to fill the booklet every day indicating any signs of menstrual bleeding and to collect the first morning urine on three pH strips. The methodology for collection and hormonal assays followed a design proposed by *Immunometrics Ltd*, London, UK. In the end, 227 women collected the urine samples and filled the booklet while 21 women returned only the booklet and the others did not participate (Rosetta L et al., submitted).

One (Q1) and two years (Q2) after recruitment Q0, the 943 eligible couples and a random sample of 817 non-eligible couples were followed-up. The eligible couples answered a questionnaire to record if the period of unprotected intercourse was finished and why (pregnancy, resuming contraception) while the non-eligible couples answered an eligibility questionnaire and were asked about any reproductive outcome occurring since the last questionnaire.

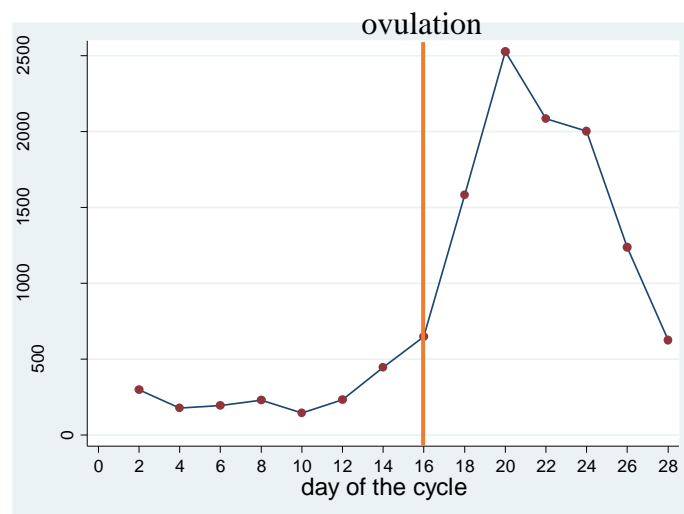
The descriptions of the recruitment protocol and of eligible women are available in Slama et al., (2012), a description of the follow-up in Duron et al., (2013) and the description of the menstrual cycle study will be submitted (Rosetta L et al., submitted). Detailed figure with the study design is available page 39 (Figure II-2)

## 2. Outcomes definition and statistical analysis

### a. Menstrual cycle characteristics

We considered the duration of each phase (follicular and luteal phases) for ovulatory cycle and considered cycle duration for all available cycles. Ovulation date was estimated using a method based on urinary values of a progesterone metabolite (pregnanediol-3- $\alpha$ -glucuronide, PdG) and creatinine measured by immunoassays (Ahsan et al., 1992) with Kassam moving averages interval method (Kassam et al., 1996; O'Connor et al., 2006). In order to identify a rise in the level of urinary PdG, this method estimates the ratio of daily PdG to the minimum 5-day moving average PdG across the cycle. The cycle is ovulatory if the ratio is more than three times the minimum 5-day moving average PdG during 3 consecutive days. This method was chosen among various other methods by being the one giving the better estimation for both every day and every other day samplings compared to estimation done by experts (considered as the gold standard method, Rosetta L et al., submitted). An example of the variations of a metabolite of progesterone in urine during one menstrual cycle and of the date of ovulation estimated by the Kassam method for one study participant is shown Figure II-1.

**Figure II-1: urinary level of a progesterone metabolite (pregnanediol-3- $\alpha$ -glucuronide, PdG) for one study participant**



Association between each air pollutant and menstrual function characteristics was estimated by linear regression adjusted on factors selected from literature. As the lengths of cycle and follicular phase were considered as censored when women did not record the first day of the next cycle (corresponding to next menses) in the booklet, those outcomes were imputed 1,000 times with age and, in case of ovulatory cycles, follicular phase length using Stata *mi impute* procedure for right-censored variables.

#### *b. Time to pregnancy studies*

We defined two outcomes, the current duration of unprotected intercourse (CDUI), relevant for a current duration analysis, and the time to pregnancy (TTP), relevant for a prevalent cohort analysis.

##### *i. Current duration design*

The current duration design included couples eligible at Q0 (women A, B, C and D in Figure II-2). Couples not eligible at Q0 were not included (women E, F, G, H and I in Figure II-2).

In the current duration design, couples were not followed-up and the only available information is the duration of unprotected intercourse from its beginning to recruitment only, called current duration of unprotected intercourse. In fecundity studies, we are interested in time to pregnancy, which is not observed in this design. Yet, from the observed distribution of the current duration of unprotected intercourse we can infer information on the unobserved distribution of the total duration of unprotected intercourse (which is different from time to pregnancy). This is possible if the model is in a stable state and if both observed and unobserved distributions follow an accelerated time model (AFT) (Keiding et al., 2011; Yamaguchi, 2003). We fitted an AFT model to estimate the association of the covariates on current duration of unprotected intercourse and, by design, on the total duration of unprotected intercourse. Results from AFT models are given as time ratios (TR. A TR of 2 correspond to a doubled median before the time of the event of interest). The TR between exposure and current duration of unprotected intercourse is equivalent to the one that show the association between exposure and the complete duration of the unprotected intercourse before pregnancy or the end of the unprotected intercourse period (Keiding et al. 2011), if the selected distribution of the underlying distribution in the AFT model is decreasing and finite in 0 (Keiding et al., 2012).

The choice between possible distributions for the AFT model was done between Exponential, Weibull, Loglogistic and Generalized Gamma (GG) distributions that are the available distributions implemented for AFT model in Stata software. For the choice of the distribution, we used goodness of fit tests (Bradburn et al., 2003; Sayehmiri et al., 2008) and we additionally checked that the two required condition are true. Only Exponential distribution fits all hypothesis.

Analyses were restricted to couples with a CDUI shorter than 36 months and to couples not resorting to medical help for infertility trouble. This allows to censor these couples (Slama et al., 2014). Adjustment factors were chosen at the start of the period of unprotected intercourse whenever possible.

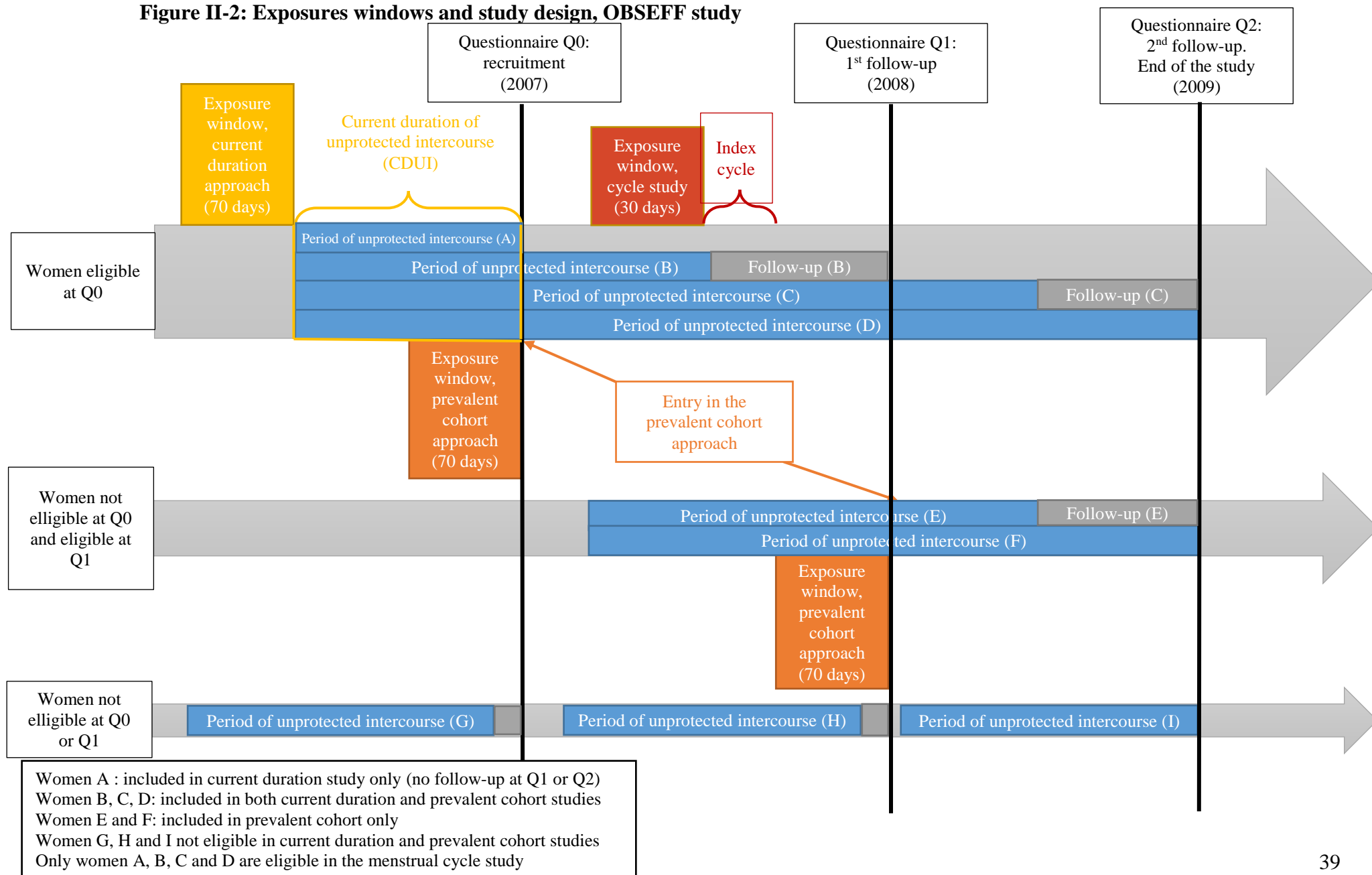
#### ii. Prevalent cohort approach

The prevalent cohort design included couples eligible at Q0 that are followed-up until Q1 (woman B in Figure II-2) or Q2 (women C and D in Figure II-2). Couples not eligible at Q0 were also included if they were eligible at Q1 and followed-up until Q2 (women E and F in Figure II-2). Women eligible at Q0 that were not followed-up (women A in Figure II-2), women eligible at Q1 but not followed-up until Q2 and women who had a period of unprotected intercourse but were not eligible at Q0 and Q1 (women G, H and I in Figure II-2) were not eligible in the prevalent cohort.

In the prevalent cohort design, couples have been followed-up for up to two years and time to pregnancy (TTP) or to censoring events can be observed. A discrete-time survival model (logistic link) with delayed entry was used to estimate hazard ratios (HR) of pregnancy. Couples eligible in current duration design were included in the analysis at Q0 with delayed entry for the period of unprotected intercourse occurring between the beginning of the period of unprotected intercourse and Q0. For the couples not eligible at Q0 and becoming eligible before the first follow-up questionnaire Q1, the entry time corresponded to the time elapsed between the start of the period of unprotected intercourse and Q1. Couples were censored when resorting to medical help for infertility trouble, after 36 months of unprotected intercourse, when the women reached 45 years old, if the period of unprotected intercourse stopped for a reason other than achieving pregnancy or at the time of the last follow-up (Q2). All adjustment factors were defined with respect to the period just before the entry date in this approach.



**Figure II-2: Exposures windows and study design, OBSEFF study**



### 3. Atmospheric pollution

#### *a. Exposure model*

The exposure model is based on the CHIMERE chemistry–transport model (Valari et al., 2011) combined with measurements from the permanent network of air quality monitoring stations allowing to obtain high temporal (hourly) and spatial (1×1 km) resolutions over an area covering France in 2009. Implementing CHIMERE model require various input data including emissions, meteorology and boundary conditions. The daily estimates for NO<sub>2</sub> and PM<sub>10</sub> were combined with hourly data from background monitoring stations with a kriging analysis (Benmerad M, in press).

#### *b. Back-extrapolation and seasonalization.*

As the urine samples were collected between October 2007 and January 2008 and as the period of unprotected intercourse considered in the fecundity study began up to 2004, we retrieved data from all the air quality monitoring stations of the country from ADEME (French Environment and Energy Management Agency) between January 2002 and July 2009. Each home address was linked to the nearest point of grid and to the nearest monitoring station at less than 100 km from home. To backextrapolate the exposures from the model to the study period, we assumed that the spatial contrast at the country scale were similar in 2009 than during the other years of the study period (2004-2008). For NO<sub>2</sub>, it was observed that the most polluted areas tends to stay more polluted through the year (Lebret et al., 2000). This procedure is not classical but can be used if the exposure model provide daily values or if data from monitoring stations are impossible to collect at the same time than the data from the exposure model.

In the case of the menstrual cycle study, we estimated the ratio of the exposure model's estimate at the home address during the 30 days of 2009 corresponding to the days and months of the exposure window divided by the exposure model's estimate at the nearest station for the same 30 days period and used this spatial ratio to correct the average during the true exposure window of the daily concentrations at the nearest station. For each exposure window, exposures of women with daily data from the station used for back-extrapolation available for less than 75% of the considered exposure window were considered missing.

To detail with the example of the menstrual cycle study, we assumed that if during a defined time period during year 2009 ( $dt_{2009}$ ) the exposure level at a location  $(X_1, Y_1)$  corresponds to  $x\%$  of the whole country average during  $dt_{2009}$ , then at the same time period during another year A ( $dt_A$ , with year A included in the study period), then exposure at the same location  $(X_1, Y_1)$  will also be  $x\%$  of the exposure in the whole country during  $dt_A$ , i.e.:

$$\frac{model_{dt_{2009}}(X_1, Y_1)}{model_{2009}(France)} = \frac{model_{dt_A}(X_1, Y_1)}{model_{dt_A}(France)} = x(X_1, Y_1) \quad (1)$$

Then, for any pairs of locations  $(X_1, Y_1)_i$  [the nearest point of the grid from the home address of woman i] and  $(X_2, Y_2)_i$  [the nearest point of the grid from the nearest station for home address of woman i] we can deduct from equation (1) that:

$$\begin{aligned} model_{dt_A}(France) &= \frac{model_{dt_A}(X_1, Y_1)_i}{model_{dt_{2009}}(X_1, Y_1)_i} model_{dt_{2009}}(France) \\ &= \frac{model_{dt_A}(X_2, Y_2)_i}{model_{dt_{2009}}(X_2, Y_2)_i} model_{dt_{2009}}(France) \\ \Leftrightarrow model_{dt_A}(X_1, Y_1)_i &= \frac{model_{dt_{2009}}(X_1, Y_1)_i}{model_{dt_{2009}}(X_2, Y_2)_i} model_{dt_A}(X_2, Y_2)_i \end{aligned} \quad (2)$$

With the exposure model with 1x1 km grid, we can estimate  $model_{dt_{2009}}(X_1, Y_1)_i$  (i.e. the exposure estimated at home address of woman i during the exposure window  $dt$  moved in 2009) and  $model_{dt_{2009}}(X_2, Y_2)_i$  (i.e. the exposure estimated at the location of nearest station from home address of woman i during the exposure window  $dt$  moved in 2009). We want to estimate  $model_{dt_i}(X_1, Y_1)_i$  (i.e. the exposure estimated at home address of woman i during the exposure window  $dt_i$  corresponding to 30 days before the index cycle of woman i), which following equation (2) corresponds to the exposure estimated at the location of the nearest station from home address of woman i during the 30 days before her index cycle (i.e.  $model_{dt_i}(X_2, Y_2)_i$ ) multiplied by a corrective factor defined during the 30 days of 2009 corresponding to the days and months of the exposure window ( $dt_{i,2009}$ ). We cannot know the exact value of  $model_{dt_i}(X_2, Y_2)_i$  as the exposure model was not available during  $dt_i$ , but we can approximate it using the values measured at the station located in  $(X_2, Y_2)_i$ . We averaged the daily data from the nearest station of home address of woman i during  $dt_i$  (average called  $station_{dt_i}(X_2, Y_2)_i$ ). Then, we estimate the exposure of woman i at her home address

during the exposure window  $dt_i$  (called  $exposure_{dt}(X_1, Y_1)_i$ ) by applying the corrective factor to the exposure estimated during  $dt_i$  with the nearest station for home address of woman  $i$ :

$$exposure_{dt_i}(X_1, Y_1)_i = \frac{model_{dt_i, 2009}(X_1, Y_1)_i}{model_{dt_i, 2009}(X_2, Y_2)_i} station_{dt}(X_2, Y_2)_i$$

### c. Exposure windows considered

For the cycle study, we considered as the main exposure window the 30 days before the index cycle (corresponding approximately to the previous cycle, Figure II-2, page 39). As a sensitivity analysis, we also considered the 90 days before the end of the cycle or the last day of the booklet.

In the fecundity study, two designs were used. In the current duration design women were recruited while not using any contraceptive method and not followed up: the exposure window was defined as the 70 days before the start of the period of unprotected intercourse to correspond to the duration of spermatogenesis (Heller and Clermont, 1964) (Figure II-2). In the prevalent cohort design, the women were followed-up and included with delayed entry to account for the fact that no event occurred between the start of the period of unprotected intercourse and entry: the exposure window in this case was defined as the 70 days before the entry (Figure II-2).

## 4. Authors contributions

R. Slama and J. Bouyer initiated the OBSEFF study. R. Slama, B. Ducot, N. Keiding, A. Bohet and J. Bouyer designed the study and questionnaires; A. Bohet., B. Ducot., J J. Bouyer. and R. Slama supervised data collection. A. Bohet, B. Ducot, L. Giorgis-Allemand and R. Slama cleaned and prepared the data. JC Thalabard estimated the day of ovulation with the Kassam method. M. Benmerad estimated the daily exposure values from the CHIMERE-adapted exposure model and L. Giorgis-Allemand estimated the exposures at the home address of study participants. L. Giorgis-Allemand and R. Slama designed the back-extrapolation procedure. L. Giorgis-Allemand did the statistical analysis.

The menstrual cycle article has been circulated among co-authors but it is not the case yet for the fecundity study.

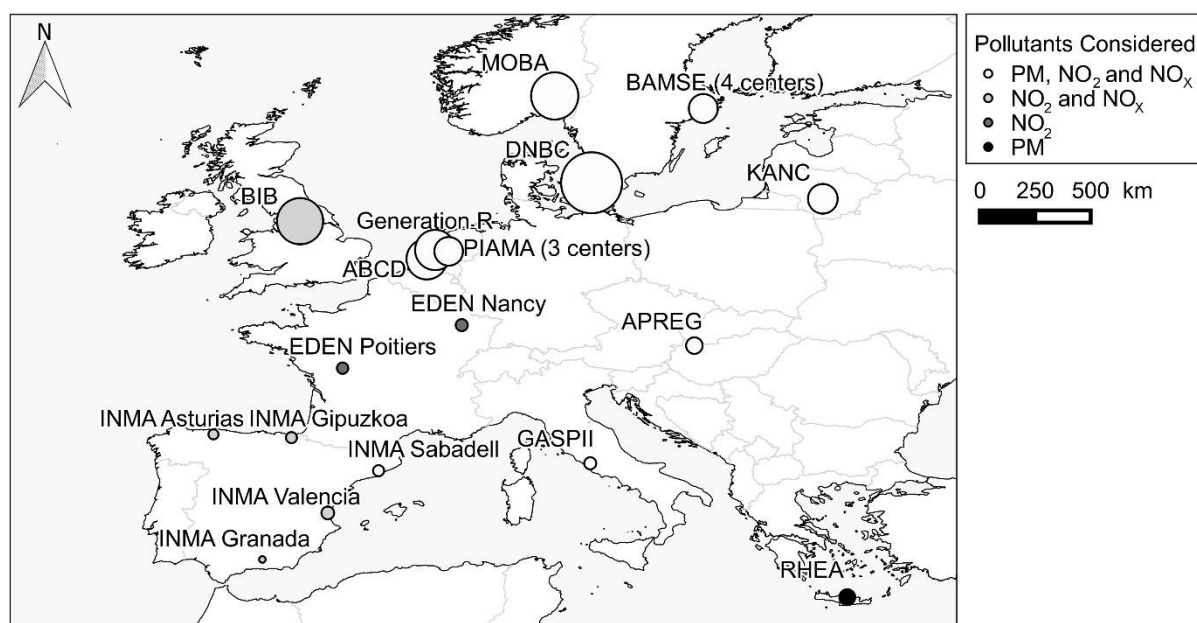
### III. ESCAPE study

The study population and exposure assessment procedure was the same as described by Pedersen et al. (2013a), except for DUISBURG cohort that was excluded because only term birth were recruited.

#### 1. Population

We considered 13 cohorts of pregnant women and newborns recruited between 1994 and 2010 in 11 European countries (ABCD, Amsterdam, The Netherlands; APREG, Gyor, Hungary; BAMSE, Stockholm area, Sweden; BiB, Bradford, England; DNBC, Copenhagen area, Denmark; EDEN, Nancy and Poitiers, France; GASPII, Rome, Italy; Generation R, Rotterdam, The Netherlands; INMA (5 centers: Asturias, Gipuzkoa, Granada, Sabadell and Valencia), Spain; KANC, Kaunas, Lithuania; MOBA, Oslo area, Norway; PIAMA (3 centers, Northern, middle and Southern part of the Netherlands) and RHEA, Heraklion, Greece) (Figure II-3). To be included, women had to have delivered a singleton live birth with known birthweight and newborn sex and to have at least one home address during pregnancy located in areas included in one of the LUR models developed within ESCAPE project. Each cohort transferred health and exposure data to Inserm (Grenoble), where harmonization and pooling were performed. Women with several pregnancies during the study period participated in the study only with the first one.

**Figure II-3: Map of cohorts included in the ESCAPE preterm delivery study**



## 2. Atmospheric pollution

### a. Exposure model

LUR models were developed following the standardized ESCAPE protocol<sup>9</sup> (Beelen et al., 2013; Eeftens et al., 2012b). For EDEN cohort, the measurement campaign was performed before the ESCAPE project (Sellier et al., 2014) but the development of the LUR model was done together with the other ESCAPE LUR models.

Briefly, three two-week measurements campaigns were performed per area. One reference background monitoring station was used to estimate yearly average of nitrogen oxides (NO<sub>2</sub>, NO<sub>x</sub>), particulate matter (PM<sub>10</sub>, PM<sub>2.5</sub>, PM<sub>10-2.5</sub>) and absorbance of PM<sub>2.5</sub>. For budgetary reason, PM and PM<sub>2.5</sub> absorbance campaigns were not performed for BiB, EDEN INMA Asturias; INMA Gipuzkoa; INMA Granada and INMA Valencia ) (Figure II-3). For each area, multiple linear regression models were constructed with a supervised stepwise selection procedure to analyze the association between concentrations and predictor variables (traffic, population density, industries, greenspaces ...). The variables selected were different for each area and were used to estimate yearly exposure at the home address(es) of each subject.

### b. Backextrapolation and seasonalization

We back-extrapolated the exposure using the ratio method described in the ESCAPE protocol<sup>10</sup>, which is similar to the procedure that have already been used in other studies (Gehring et al., 2011a; Lepeule et al., 2010; Slama et al., 2007). To use this procedure, station data must be available during the year in which the annual LUR model was estimated and during the pregnancies of the study's participants. The main assumption of this method is that exposures from the model have the same temporal variations than one representative background station from the same area.

With  $dt_1$  and  $dt_2$  two different periods of time, and yearly exposure of the LUR model known for  $dt_2$ , then we assume that the ratio of exposures measured by the LUR model and the nearest station during  $dt_1$  and  $dt_2$  are identical:

$$\frac{station_{dt1}}{station_{dt2}} = \frac{model_{dt1}}{model_{dt2}}$$

<sup>9</sup> <http://www.escapeproject.eu/manuals/index.php>

<sup>10</sup> [http://www.escapeproject.eu/manuals/Procedure\\_for\\_extrapolation\\_back\\_in\\_time.pdf](http://www.escapeproject.eu/manuals/Procedure_for_extrapolation_back_in_time.pdf)

Then we can estimate exposure with the LUR model during  $dt_2$  by:

$$model_{dt1} = model_{dt2} \frac{station_{dt1}}{station_{dt2}}$$

When air quality monitoring data from a background station was unavailable for a given pollutant (which happened in some cohorts for PM<sub>10</sub>, PM<sub>2.5</sub> and PM<sub>2.5</sub> absorbance), we used measures for another pollutant during the same time period as a replacement. The choice of the pollutant used to back extrapolate another pollutant was based on an extensive study of temporal correlations between pollutants simultaneously available in each area: NO<sub>x</sub> background data were used to estimate the temporal component for PM<sub>2.5</sub> absorbance (MoBa, BAMSE, DNBC, KANC, APREG, GASPII and INMA-Sabadell cohorts). When PM<sub>10</sub> background data were unavailable, we used the temporal component from total suspended particles (DNBC) or from NO<sub>2</sub> (INMA-Sabadell); finally, when PM<sub>2.5</sub> measures were unavailable, we used the PM<sub>10</sub> temporal component (BAMSE, ABCD, Generation R, PIAMA, APREG and GASPII) or, if PM<sub>10</sub> were not available, the same pollutant that we had applied for PM<sub>10</sub> backextrapolation (DNBC and INMA-Sabadell).

#### *c. Exposure windows considered*

We considered 1<sup>st</sup> trimester of pregnancy (from day 14 – counting from the last menstrual period – to day 105), 2<sup>nd</sup> trimester (from day 106 to day 197), whole pregnancy (from day 14 to birth or day 259, whichever came first), last week and last 4-weeks (see Figure I-7, page 21).

For each exposure window, exposures were considered missing if pregnant women lived in the study area during less than 75% of the considered time period or if exposures of women were estimated with daily data from the station used for back-extrapolation for less than 75% of the considered time period.

#### *d. Residential mobility during pregnancy*

Whenever possible, we considered the residential mobility during pregnancy by pondering the exposures using the proportion of time spend at each address: for some cohorts (BAMSE, DNBC, Generation R, EDEN and APREG), information on date of moving during pregnancy was available and we estimated an exposure variable taking into account the multiple addresses. As very few women moved more than twice during pregnancy, we only considered

the first two addresses where the women lived for at least one week. Exposure of women who lived in the study area (defined by the areas covered by LUR models) during less than 75% of the considered time period were considered as missing. For the remaining cohorts, only one address was known and date of moving (if any) was unknown, although for some of the cohorts (ABCD, BIB, GASPII, INMA, KANC, MoBa, PIAMA), we knew if pregnant women moved during pregnancy, but we had insufficient information to take this into account; in these cohorts and cohorts with no information on maternal mobility during pregnancy (MoBa and RHEA), exposures were estimated as if women had not moved. Among cohorts with only one address, only the first address at the time of recruitment some time before birth was used for MoBa, ABCD, PIAMA, INMA and RHEA, while the birth address was used for KANC, BiB, PIAMA and GASPII.

### 3. Meteorological parameters

Outdoor temperature, humidity and atmospheric pressure at the altitude of the city were defined from the daily measures of one monitoring station by center and averaged during the same exposure windows than atmospheric pollutants. For BAMSE and DNBC cohorts, as the research team in charge of the cohort could not provide data for atmospheric pressure, we used the European Climate Assessment Dataset<sup>11</sup> and downloaded data from the nearest station identified in the database. Atmospheric pressure was not available for KANC cohort, with no information available in the European Climate Assessment Dataset for Lithuania at the right time period. When atmospheric pressure was available only at sea level (EDEN, BIB, GASPII, BAMSE) or if the monitoring station was not in the city (DNBC), we used the following formula to correct the altitude assuming that the atmosphere is behaving as an ideal gas by using the barometric formula adapted by meteorologists<sup>12</sup>:

$$P_{station} = \frac{P_{mer}}{\left(\frac{T_{station} + \alpha H}{T_{station}}\right)^{\frac{Mg}{R\alpha}}}$$

With:  $T_{station}$  temperature measured by the monitoring station in Kelvins ( $=C^{\circ}+273.15$ )

$H$  altitude of the monitoring station in meters

$\alpha$  temperature lapse rate (constant=0.0065 K.m<sup>-1</sup>)

$M$  molar mass of air (0.029 kg.mol<sup>-1</sup>)

$g$  Earth-surface gravitational acceleration (9.81 m.s<sup>-2</sup>)

$R$  ideal gas constant (8.31 J.K<sup>-1</sup>mol<sup>-1</sup>)

<sup>11</sup> <http://www.ecad.eu/dailydata/customquery.php>

<sup>12</sup> <http://www.deleze.name/~marcel/sec2/applmaths/pression-altitude/pression-altitude.pdf>



#### 4. Preterm birth definition and statistical analysis

Preterm births are birth occurring before 37 completed gestational weeks. Whenever possible, we considered gestational duration based on conception date estimated from the last menstrual period. For cohorts (or births) in which this information was not available, we used by order of decreasing preference the ultrasound-based estimate or gestational duration from birth records. When the discrepancy between last menstrual period-based gestational duration (or the information from birth records) and the ultrasound-based estimate was three weeks or more, we modified values assuming the ultrasound-based estimate was correct.

Analyses of preterm birth risk were conducted pooling all cohorts with a random effect for study center. Associations during first and second trimesters were estimated using adjusted logistic regressions while associations during whole pregnancy, last week and last month were estimated with a discrete time survival model (logistic link) with time-varying exposures censored at 37 gestational weeks.

#### 5. Authors contribution

The following authors have been implied in definition of the cohort protocol, meteorological and health and confounder data collection as well as air pollution field measurements: M van Eijsden, T G. M. Vrijkotte (ABCD cohort), M J. Varró, P Rudnai (APREG cohort), M Korek, G Pershagen (BAMSE cohort), D Tuffnell, J Wright (BiB cohort), K Thorup Eriksen, O Raaschou-Nielsen, M Sørensen (DNBC cohort), C Bernard, L Giorgis-Allemand, B Heude, J Lepeule, , R Slama (Eden cohort), F Forastiere, D Porta (GASPII), E H. van den Hooven, V Jaddoe (Generation R cohort), I Aguilera, M Cirach, J Sunyer (Inma Sabadell cohort), C Iñiguez, M Estarlich (Inma Valencia cohort), A Fernández-Somoano (Inma Asturias cohort), M F. Fernández (Inma Granada cohort), A Lertxundi (Inma Gipuzkoa), A Danileviciute, A Dedele, R Grazuleviciene (KANC cohort); S E. Håberg, P Nafstad, W Nystad (MOBA), U Gehring, D Postma, A Wijga (PIAMA cohort) M Kogevinas, E Patelarou, E Stephanou and L Chatzi (RHEA cohort).

A first version of the study analysis protocol has been prepared by J Lepeule, E Patelarou, M Kogevinas and R Slama. The data preparation has implied all authors; data harmonization and pooling has been done by C Bernard, L Giorgis-Allemand, M Pedersen and R Slama; estimation of exposure during pregnancy by L Giorgis-Allemand and R Slama. The birth outcome working group of Escape project was led by R Slama and M Kogevinas. The birth

cohort work package of Escape was coordinated by G Pershagen with the support of O Gruzieva; the exposure work package by G Hoek with support from R Beelen and K de Hoogh, while B Brunekreef was P.I. of ESCAPE project. Statistical analyses have been done by L Giorgis-Allemand, C Bernard and R Slama. The manuscript has been drafted by R Slama and L Giorgis-Allemand. V Siroux provided critical comments on the statistical analyses and the manuscript. All authors of the paper have contributed to critical review of the preterm birth article.



## Chapter III: Before fertilization: Atmospheric pollution and menstrual cycle



## I. French summary

**Introduction :** Quelques études ont suggéré que la pollution atmosphérique pourrait avoir un impact sur la fertilité (la capacité biologique à concevoir pour un couple) ou sur les paramètres masculins de la fertilité (paramètres spermatiques). A notre connaissance, la fertilité féminine n'a quant à elle pas encore été étudiée.

**Objectif :** Notre but était de caractériser l'influence à court terme de la pollution atmosphérique sur le cycle menstruel de femmes recrutées en population générale.

**Méthodes :** Nous avons recruté en population générale un échantillon aléatoire de 250 femmes n'utilisant aucune méthode contraceptive. Pendant un cycle menstruel, les femmes ont recueilli chaque jour -ou un jour sur deux- la première urine de la journée sur des bandelettes de papier pH tout en complétant un cahier journalier dans lequel elles devaient indiquer leurs menstruations. Un dérivé de la progestérone (Pregnanediol-3 $\alpha$ -Glucuronide) a été mesuré par une méthode immuno-enzymatique (Ahsan et al., 1992). Un algorithme de détection a été utilisé pour définir le jour de l'ovulation (Kassam et al., 1996), durée de la phase folliculaire (du premier jour des menstruation jusqu'au jour de l'ovulation) et la durée de la phase lutéale (du jour suivant l'ovulation jusqu'à la fin du cycle).

Un modèle de pollution atmosphérique a été saisonnalisé puis back-extrapolé à l'aide de la station la plus proche du domicile pour estimer les niveaux de dioxyde d'azote (NO<sub>2</sub>) et de particules fines (PM<sub>10</sub>) durant les 30 jours précédant le cycle.

Les associations entre la pollution atmosphérique et les durées du cycle et des phases folliculaires et lutéales ont été étudiées par des régressions linéaires. Dans le cas du cycle et de la phase lutéale, les valeurs censurées (carnets arrêtés avant les menstruations suivantes) ont été imputées 1000 fois en fonction de l'âge et de la durée de la phase folliculaire. Les analyses ont été ajustées sur des facteurs sélectionnés dans la littérature.

**Résultats :** La durée médiane du cycle était de 29 jours (n=127, 5<sup>ème</sup>-95<sup>ème</sup> percentiles : 23-40) avec une phase folliculaire durant en médiane 19 jours (n=162, 14-27) et une phase lutéale de 10 jours (n=117, 6-14). Les niveaux de NO<sub>2</sub> et PM<sub>10</sub> durant le mois précédant le cycle étudié ont été définis pour 181 femmes avec une moyenne (5<sup>ème</sup>-95<sup>ème</sup> percentiles) de respectivement 30  $\mu\text{g}/\text{m}^3$  (16-48) pour le NO<sub>2</sub> et 20  $\mu\text{g}/\text{m}^3$  (13-29) pour les PM<sub>10</sub>. Pour chaque augmentation de 10  $\mu\text{g}/\text{m}^3$  de NO<sub>2</sub>, la durée de la phase folliculaire augmentait de 0.7 jours (intervalle de confiance -IC- à 95% : [0.2;1.3]) (respectivement pour les PM<sub>10</sub>: 1.6 jours, IC

95% [0.3;2.9]). Les résultats étaient assez robustes quand les analyses étaient restreintes aux femmes déclarant avoir un cycle régulier ou vivant à moins de 10 km d'une station de mesure.

Discussion: Les niveaux de NO<sub>2</sub> et PM<sub>10</sub> durant les 30 jours précédant un cycle menstruel étaient associés avec une augmentation de la durée de la phase folliculaire durant le cycle suivant. Notre étude basée sur des mesures biologiques des caractéristiques du cycle menstruel suggère que celles-ci peuvent être altérées par la pollution atmosphérique.

II. [Research letter, to be submitted to Epidemiology](#)

**Atmospheric pollution and characteristics of the menstrual cycle.**

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## **Introduction**

The literature on the possible influence of atmospheric pollution on human biological ability to conceive, or fecundity, is scarce. Regarding females markers of fecundity, longer estrous cycle and estrus were reported in mice exposed to non-filtered ambient air (Veras et al., 2009). To our knowledge, no study was conducted in humans to investigate a possible effect of atmospheric pollution on menstrual cycle characteristics. We therefore aimed to characterize any short-term influence of atmospheric pollutants on the menstrual cycle characteristics of women from the general population.

## **Methods**

We drew our study population from the OBSEFF study, a transversal sample of couples from the French general population not using any contraceptive methods (Slama et al., 2012). A subsample of 228 women participated in an ancillary study by filling daily diaries and impregnating paper strips with urine samples at least every other day during one menstrual cycle. Urinary values of pregnanediol-3- $\alpha$ -glucuronide, a progesterone metabolite, and creatinine were measured by immunoassays (Ahsan et al., 1992) and ovulation date was estimated using a moving averages interval method (Kassam et al., 1996; O'Connor et al., 2006). Exposures to nitrogen dioxide (NO<sub>2</sub>) and particulate matter with diameter below 10  $\mu$ m (PM<sub>10</sub>) at the residential address were estimated by combining estimates from an exposure model with 1 km spatial resolution and measurements from the nearest background monitor (see eAppendix). We a priori considered exposure windows of 30 days before the menstrual cycle start. We suspected that 41 women did not collect urine until the next menses without being pregnant and we used multiple imputation based on age and (for ovulatory cycles) follicular phase duration to impute missing cycle and luteal phase lengths. Linear regressions were used to study the association between air pollutants and follicular phase, luteal phase and cycle lengths. Adjustments factors were chosen amongst potential confounders selected from literature (see Table III-1). Several sensitivity analyses were conducted, including considering an exposure window of 90 days before the end of the index cycle.

The study was done in accordance with the French regulation on research on human subjects.

## **Results**

Menstrual cycle characteristics were defined for 184 women. Mean follicular phase length was 19 days (n=162), mean luteal phase length 10 days (n=117) and mean cycle length 29 days (n=127). A flow-chart and detailed participants' characteristics are given in the eAppendix. NO<sub>2</sub> and PM<sub>10</sub> levels during the 30 days before the cycle were estimated for 181 women; median values (5<sup>th</sup>-95<sup>th</sup> percentiles) were 29 µg/m<sup>3</sup> (16-48) for NO<sub>2</sub> and 20 µg/m<sup>3</sup> (13-29) for PM<sub>10</sub> with a correlation between both levels of 0.5. For each increase by 10 µg/m<sup>3</sup>, mean follicular length increased by 0.7 day (95% Confidence Interval (CI) 0.2; 1.3) with NO<sub>2</sub>; and by 1.6 day (95% CI: 0.3; 3.0) with PM<sub>10</sub>. Results were quite robust to restriction to women declaring to have regular cycles or living less than 10 km away from a monitoring station (Table III-1) or other sensitivity analysis (eAppendix). No association was observed with luteal phase length (p>0.1). No association was observed for neither outcomes with the 90 days exposure window before the end of the index cycle.

## **Discussion**

Our study based on biological measures of several menstrual cycle characteristics suggests a short-term influence of atmospheric pollution on cycle characteristics and in particular follicular phase length. NO<sub>2</sub> and PM<sub>10</sub> levels in the 30 days before the cycle were associated with an increased follicular phase duration. A study strength was the ability to include women from the whole country and to rely on biological measures. The method used to estimate day of ovulation might introduce an error in the estimation of the length of follicular phase as it is based in identifying a rise in the urinary progesterone metabolite corrected by creatinine but results were similar using definition of ovulation by an expert. These original results call for more research on air pollutants effects on menstrual cycle characteristics.

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**Table III-1: Adjusted change in the duration of cycle, follicular and luteal phase associated with atmospheric pollution levels (n=181)**

Exposure and analysis <sup>a</sup>	Follicular phase length				Luteal phase length <sup>b</sup>				Cycle length <sup>c</sup>			
	N	$\beta$	95% CI	p-value	N	$\beta$	95% CI	p-value	N	$\beta$	95% CI	p-value
<b>Single pollutant model</b>												
<b>NO<sub>2</sub>, 30 days before the start of the index cycle</b>												
Continuous*	159	0.74	0.15;1.33	0.01	144	0.28	-0.19;0.75	0.24	165	0.53	-0.48;1.54	0.30
Lowest tertile (6-18 $\mu\text{g}/\text{m}^3$ )	38	0.00	reference	0.06**	36	0.00	reference	0.12**	44	0.00	reference	<0.01**
Medium tertile (18-30 $\mu\text{g}/\text{m}^3$ )	51	0.21	-1.47;1.89		46	0.35	-0.83;1.53		53	-1.93	-5.18;1.32	
Highest tertile (30-61 $\mu\text{g}/\text{m}^3$ )	70	1.41	-0.16;2.98		62	1.08	-0.30;2.45		68	1.14	-1.80;4.07	
<b>Sensitivity analyses restricted to</b>												
Regular cycles only*	125	0.83	0.17;1.49	0.01	118	0.37	-0.11;0.85	0.13	130	1.04	0.12;1.96	0.03
No pregnancy in last 2 years*	107	1.18	0.51;1.84	<0.01	98	0.18	-0.45;0.81	0.57	112	1.11	-0.37;2.59	0.14
Adjusted on season*	159	0.80	0.23;1.36	0.01	144	0.29	-0.18;0.77	0.23	165	0.54	-0.53;1.60	0.32
Urine samples every day*	34	0.28	-1.46;2.02	0.74	30	-0.15	-0.97;0.67	0.69	34	-1.26	-2.82;0.29	0.10
Urine samples every other day*	125	0.70	0.05;1.36	0.04	114	0.25	-0.31;0.81	0.37	131	0.47	-0.76;1.71	0.45
Nulliparous women only*	62	0.87	0.01;1.72	0.05	56	0.43	-0.29;1.14	0.24	69	1.08	-0.66;2.82	0.22
Stations<10 km*	104	0.83	-0.01;1.67	0.05	92	-0.14	-0.73;0.45	0.63	102	0.80	-0.44;2.03	0.20
<b>NO<sub>2</sub>, 90 days before the end of the index cycle<sup>d</sup></b>												
Continuous*	136	0.34	-0.29;0.96	0.29	122	0.40	-0.05;0.85	0.08	139	0.24	-0.82;1.31	0.65
Lowest tertile (6-18 $\mu\text{g}/\text{m}^3$ )	33	0.00	reference	0.55**	30	0.00	reference	0.24**	37		reference	1.00**
Medium tertile (18-29 $\mu\text{g}/\text{m}^3$ )	44	-0.38	-2.29;1.52		41	0.65	-0.56;1.86		45	-2.43	-5.93;1.08	
Highest tertile (29-55 $\mu\text{g}/\text{m}^3$ )	59	0.41	-1.32;2.13		51	0.83	-0.48;2.15		57	0.35	-2.52;3.21	
<b>PM<sub>10</sub>, 30 days before the start of the index cycle</b>												
Continuous*	158	1.60	0.27;2.92	0.02	143	-0.21	-1.34;0.93	0.72	165	0.44	-1.63;2.51	0.68
Lowest tertile (11-17 $\mu\text{g}/\text{m}^3$ )	42	0.00	reference	0.02**	39	0.00	reference	0.93**	43	0.00	reference	1.00**
Medium tertile (17-21 $\mu\text{g}/\text{m}^3$ )	54	1.68	0.17;3.19		48	-0.02	-1.27;1.23		60	2.30	-0.87;5.47	
Highest tertile (21-33 $\mu\text{g}/\text{m}^3$ )	62	1.87	0.42;3.32		56	0.06	-1.29;1.40		62	0.94	-2.01;3.89	
<b>Sensitivity analyses restricted to</b>												
Regular cycles only*	124	1.61	-0.19;3.41	0.08	117	0.29	-0.89;1.47	0.62	130	1.70	-0.31;3.72	0.10
No pregnancy in last 2 years*	106	1.95	0.38;3.52	0.02	97	-0.52	-2.21;1.18	0.55	112	0.39	-2.85;3.64	0.81
Adjusted on season*	158	1.45	0.17;2.72	0.03	143	-0.21	-1.34;0.93	0.72	165	0.46	-1.59;2.52	0.66
Urine samples every day*	34	0.89	-2.38;4.16	0.58	30	-0.84	-3.01;1.33	0.41	34	-1.77	-4.66;1.12	0.21
Urine samples every other day*	124	1.79	0.18;3.41	0.03	113	0.05	-1.25;1.34	0.94	131	0.78	-1.84;3.40	0.56
Nulliparous women only*	61	1.74	-0.44;3.93	0.12	55	-0.24	-1.93;1.45	0.78	69	0.95	-2.47;4.38	0.58
Stations<10 km*	89	1.27	-0.47;3.02	0.15	77	-0.59	-1.83;0.66	0.35	85	-0.05	-2.41;2.31	0.97
<b>PM<sub>10</sub>, 90 days before the end of the index cycle<sup>d</sup></b>												
Continuous*	136	-0.22	-1.83;1.39	0.79	122	-0.13	-1.88;1.61	0.88	140	-1.77	-5.03;1.49	0.28
Lowest tertile (13-18)	35	0.00	reference	0.81**	30	0.00	reference	0.80**	36	0.00	reference	<0.01**
Medium tertile (18-21)	44	0.52	-1.34;2.38		41	0.69	-0.69;2.06		48	-0.86	-4.97;3.24	
Highest tertile (21-30)	57	0.15	-1.26;1.56		51	0.19	-1.21;1.60		56	-1.55	-4.78;1.67	
<b>Two pollutant models</b>												
<b>Exposures 30 days before start of the index cycle</b>												
NO <sub>2</sub> *	158	0.52	-0.12;1.16	0.11	143	0.41	-0.11;0.93	0.12	164	0.52	-0.62;1.65	0.37
PM <sub>10</sub> *	158	0.99	-0.50;2.47	0.19	143	-0.65	-1.86;0.55	0.28	164	-0.06	-2.33;2.22	0.96
<b>Exposures 90 days before the end of the index cycle<sup>d</sup></b>												
NO <sub>2</sub> *	136	0.43	-0.28;1.14	0.23	122	0.49	-0.01;0.99	0.06	139	0.55	-0.52;1.62	0.31
PM <sub>10</sub> *	136	-0.82	-2.65;1.00	0.37	122	-0.79	-2.69;1.11	0.41	139	-2.59	-5.78;0.59	0.11

\* : Changes in mean duration associated with an increase by 10  $\mu\text{g}/\text{m}^3$  in air pollution level.

\*\* : p-value for trend across exposure tertiles

<sup>a</sup>: Linear regression adjusted on woman age (<30; 30-34; 35-39; 40-45 years), body mass index (<25,  $\geq$ 25 kg/m<sup>2</sup>, 3 missing values imputed in the lowest category), age at menarche (8-12; 12-18 years, 3 missing values imputed in the higher category), parity (nulliparous/parous), alcohol consumption in the week before inclusion (yes/no), caffeine consumption in the week before inclusion (yes/no), active smoking at inclusion (yes/no), passive smoking at inclusion (yes/no), education level (3 categories) and professional activity (yes/no)<sup>b</sup>: Missing values for luteal phase (n=29) were imputed 1000 times using age and follicular length.<sup>c</sup>: Missing values for cycle (n=41) were imputed 1000 times using age and follicular length is the cycle is ovulatory, age only if anovulatory.<sup>d</sup>: 90 days before the end of the index cycle (only available for women with observed menses at the end of the index cycle)

III. eAppendix

**Summary**

Acknowledgments and fundings	60
Details of exposure estimation	61
Table S III-1: Characteristics of the study population (n=184)	63
Table S III-2: Adjusted association between menstrual cycle, follicular phase and luteal phase lengths and atmospheric pollution, analyses restricted to 138 women with regular cycles	64
Table S III-3: Adjusted association between phase lengths and atmospheric pollution, using date of ovulation defined by an expert instead of the Kassam method	65
Figure S III-1: Flow-chart	66
Figure S III-2: Distribution of outcomes	67
Figure S III-3: Location of the home addresses of the study participants, and yearly NO <sub>2</sub> levels in France from the chemical-transport model during year 2009	68

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### Estimation of exposure to atmospheric pollution

A fine spatial exposure model for NO<sub>2</sub> and PM<sub>10</sub> with 1x1 km spatial resolution and daily temporal resolution was developed by INERIS (French national institute for industrial environment and risks), covering the whole country for years 2009-2010. The model was developed by kriging daily data from the CHIMERE chemistry-transport model and using as drift daily data from air quality monitors and -for NO<sub>2</sub>- NO<sub>x</sub> emission inventory (Benmerad M, *in press*).

As the urine samples were collected between October 2007 and January 2008 and as we were interested in the exposures in the 30 days before the start of the cycle and the 90 days before the end of the cycle, we retrieved data from all the air quality monitoring stations of the country from ADEME (French Environment and Energy Management Agency) between January 2002 and July 2009. Each home address was linked to the nearest point of grid and to the nearest monitoring station at less than 100 km from home. To backextrapolate the exposures from the model to the study period, we assumed that the spatial contrast at the country scale were similar in 2009 than during the other years of the study period (2007-2008). We estimated the ratio of the exposure model's estimate at the home address during the 30 days of 2009 corresponding to the days and months of the exposure window divided by the exposure model's estimate at the nearest station for the same 30 days period and used this spatial ratio to correct the average during the true exposure window of the daily concentrations at the nearest station. For each exposure window, exposures of women with daily data from the station used for back-extrapolation available for less than 75% of the considered exposure window were considered missing.

To detail, we assumed that if during a defined time period during year 2009 (dt<sub>2009</sub>) the exposure level at a location (X<sub>1</sub>,Y<sub>1</sub>) corresponds to x% of the whole country average during dt<sub>2009</sub>, then at the same time period during another year A (dt<sub>A</sub>, with year A included in the study period), then exposure at the same location (X<sub>1</sub>,Y<sub>1</sub>) will also be x% of the exposure in the whole country during dt<sub>A</sub>, i.e.:

$$\frac{model_{dt_{2009}}(X_1, Y_1)}{model_{2009}(France)} = \frac{model_{dt_A}(X_1, Y_1)}{model_{dt_A}(France)} = x(X_1, Y_1) \quad (1)$$

Then, for any pairs of locations  $(X_1, Y_1)_i$  [the nearest point of the grid from the home address of woman  $i$ ] and  $(X_2, Y_2)_i$  [the nearest point of the grid from the nearest station for home address of woman  $i$ ] we can deduct from equation (1) that:

$$\begin{aligned} model_{dt_A}(France) &= \frac{model_{dt_A}(X_1, Y_1)_i}{model_{dt_{2009}}(X_1, Y_1)_i} model_{dt_{2009}}(France) \\ &= \frac{model_{dt_A}(X_2, Y_2)_i}{model_{dt_{2009}}(X_2, Y_2)_i} model_{dt_{2009}}(France) \\ \Leftrightarrow model_{dt_A}(X_1, Y_1)_i &= \frac{model_{dt_{2009}}(X_1, Y_1)_i}{model_{dt_{2009}}(X_2, Y_2)_i} model_{dt_A}(X_2, Y_2)_i \quad (2) \end{aligned}$$

With the exposure model with 1x1 km grid, we can estimate  $model_{dt_{2009}}(X_1, Y_1)_i$  (i.e. the exposure estimated at home address of woman  $i$  during the exposure window  $dt$  moved in 2009) and  $model_{dt_{2009}}(X_2, Y_2)_i$  (i.e. the exposure estimated at the location of nearest station from home address of woman  $i$  during the exposure window  $dt$  moved in 2009). We want to estimate  $model_{dt_i}(X_1, Y_1)_i$  (i.e. the exposure estimated at home address of woman  $i$  during the exposure window  $dt_i$  corresponding to 30 days before the index cycle of woman  $i$ ), which following equation (2) corresponds to the exposure estimated at the location of the nearest station from home address of woman  $i$  during the 30 days before her index cycle (i.e.  $model_{dt_i}(X_2, Y_2)_i$ ) multiplied by a corrective factor defined during the 30 days of 2009 corresponding to the days and months of the exposure window ( $dt_{i,2009}$ ). We cannot know the exact value of  $model_{dt_i}(X_2, Y_2)_i$  as the exposure model was not available during  $dt_i$ , but we can approximate it using the values measured at the station located in  $(X_2, Y_2)_i$ . We averaged the daily data from the nearest station of home address of woman  $i$  during  $dt_i$  (average called  $station_{dt_i}(X_2, Y_2)_i$ ). Then, we estimate the exposure of woman  $i$  at her home address during the exposure window  $dt$  (called  $exposure_{dt}(X_1, Y_1)_i$ ) by applying the corrective factor to the exposure estimated during  $dt_i$  with the nearest station for home address of woman  $i$ :

$$exposure_{dt_i}(X_1, Y_1)_i = \frac{model_{dt_{i,2009}}(X_1, Y_1)_i}{model_{dt_{i,2009}}(X_2, Y_2)_i} station_{dt_i}(X_2, Y_2)_i$$

### References:

Benmerad M (in press). Chronic effects of air pollution on lung function after lung transplantation in the Systems prediction of Chronic Lung Allograft Dysfunction (SysCLAD) study. ERJ.



**Table S III-1: Characteristics of the study population (n=184)**

Characteristics	N	%	%*	Mean	Percentiles		
					5 <sup>th</sup>	50 <sup>th</sup>	95 <sup>th</sup>
Cycle duration (days)	127			28.7	23	28	40
Follicular phase (days)	162			19.1	14	18	27
Luteal phase (days)	117			9.8	6	10	14
Ovulatory cycle							
No	19	10	13				
Yes	165	90	87				
Pregnancy occurring during index cycle							
No	166	90	92				
Yes	16	9	7				
Unknown	2	1	1				
Age (years)				34	26	35	43
< 30	33	18	24				
30-34	52	28	29				
35-39	61	33	26				
>40	38	21	21				
Body Mass Index (kg/m <sup>2</sup> )				23	18	23	34
Underweight (<18.5)	12	7	9				
Normal range (18.5-25)	125	68	61				
Overweight (25-30)	31	17	19				
Obese (>30)	13	7	7				
Missing	3	2	3				
Age at menarche*** (years)	178			13	10	13	16
Nulliparous							
No	105	57	54				
Yes	79	42	46				
Drink more than one glass of coffee per day							
No	72	39	41				
Yes	112	61	59				
Active smoker at inclusion							
No	135	73	70				
Yes	49	27	30				
Passive smoker at inclusion							
No	121	66	56				
Yes	63	34	44				
Education level							
<bac	26	14	26				
bac	63	34	39				
>bac	95	52	35				
Employed							
No	41	22	25				
Yes	143	78	75				
NO <sub>2</sub>							
30 days before the start of the index cycle	180			30.3	16.1	29.0	47.6
90 days before the end of the index cycle	153			30.1	15.6	28.8	48.5
PM <sub>10</sub>							
30 days before the start of the index cycle	180			19.5	12.9	19.6	28.5
90 days before the end of the index cycle	154			20.2	15.7	20.3	25.1

\* Percentages were corrected for possible selection bias and over-representation of urban compared with rural areas.

**Table S III-2: Adjusted association between menstrual cycle, follicular phase and luteal phase lengths and atmospheric pollution, analyses restricted to 138 women with regular cycles**

Exposure and analysis <sup>a</sup>	Follicular phase length				Luteal phase length <sup>b</sup>				Cycle length <sup>c</sup>			
	N	$\beta^*$	95% CI	p-value	N	$\beta^*$	95% CI	p-value	N	$\beta^*$	95% CI	p-value
<b>NO<sub>2</sub>, 30 days before the start of the index cycle</b>												
Continuous	125	0.83	0.17;1.49	0.01	118	0.37	-0.11;0.85	0.13	130	1.04	0.12;1.96	0.03
<b>Sensitivity analyses restricted to</b>												
No pregnancy in last 2 years	83	1.31	0.61;2.00	<0.01	80	0.16	-0.44;0.76	0.60	87	2.02	0.68;3.36	<0.01
Adjusted on season	125	0.88	0.21;1.54	0.01	118	0.38	-0.10;0.87	0.12	130	1.04	0.10;1.98	0.03
Nulliparous women only	44	0.91	-0.20;2.02	0.11	42	0.32	-0.34;0.99	0.33	48	1.69	0.10;3.28	0.04
Stations<10 km	80	0.67	-0.36;1.69	0.20	74	0.12	-0.48;0.71	0.69	79	0.70	-0.32;1.72	0.17
<b>NO<sub>2</sub>, 90 days before the end of the index cycle<sup>d</sup></b>												
Continuous	105	0.41	-0.27;1.09	0.24	99	0.35	-0.12;0.81	0.14	110	0.51	-0.41;1.43	0.28
<b>PM<sub>10</sub>, 30 days before the start of the index cycle</b>												
Continuous	124	1.61	-0.19;3.41	0.08	117	0.29	-0.89;1.47	0.62	130	1.70	-0.31;3.72	0.10
<b>Sensitivity analyses restricted to</b>												
No pregnancy in last 2 years	82	2.05	0.07;4.03	0.04	79	0.17	-1.44;1.78	0.83	87	2.06	-0.53;4.65	0.12
Adjusted on season	124	1.54	-0.26;3.34	0.09	117	0.29	-0.89;1.47	0.63	130	1.73	-0.34;3.80	0.10
Nulliparous women only	43	1.07	-1.83;3.98	0.46	41	0.47	-0.94;1.88	0.50	48	1.80	-0.77;4.37	0.16
Stations<10 km	68	0.17	-2.35;2.68	0.89	62	0.20	-1.09;1.48	0.76	65	-0.32	-2.44;1.80	0.76
<b>PM<sub>10</sub>, 90 days before the end of the index cycle<sup>d</sup></b>												
Continuous	105	0.11	-2.01;2.23	0.92	99	0.22	-1.55;1.99	0.80	111	-0.42	-4.21;3.38	0.83

\*: Changes in mean duration associated with an increase by 10  $\mu\text{g}/\text{m}^3$  in air pollution level.

<sup>a</sup>: Linear regression adjusted on woman age (<30; 30-34; 35-39; 40-45 years), body mass index (<25,  $\geq$ 25  $\text{kg}/\text{m}^2$ , 3 missing values imputed in the lowest category), age at menarche (8-12; 12-18 years, 3 missing values imputed in the higher category), parity (nulliparous/parous), alcohol consumption in the week before inclusion (yes/no), caffeine consumption in the week before inclusion (yes/no), active smoking at inclusion (yes/no), passive smoking at inclusion (yes/no), education level (3 categories) and professional activity (yes/no)

<sup>b</sup>: Missing values for luteal phase (n=24) were imputed 1000 times using age and follicular length.

<sup>c</sup>: Missing values for cycle (n=29) were imputed 1000 times using age and follicular length is the cycle is ovulatory, age only if anovulatory.

<sup>d</sup>: 90 days before the end of the index cycle (only available for women with observed menses at the end of the index cycle)

**Table S III-3: Adjusted association between phase lengths and atmospheric pollution, using date of ovulation defined by an expert instead of the Kassam method**

Exposure and analysis <sup>a</sup>	Follicular phase length				Luteal phase length <sup>b</sup>			
	N	$\beta^*$	95% CI	p-value	N	$\beta^*$	95% CI	p-value
<b>NO<sub>2</sub>, 30 days before the start of the index cycle</b>								
Continuous	157	0.81	0.29;1.33	<0.01	144	0.21	-0.28;0.71	0.40
<b>Sensitivity analyses restricted to</b>								
Regular cycles	124	0.80	0.18;1.42	0.01	118	0.39	-0.13;0.91	0.14
No pregnancy in last 2 years	107	1.23	0.61;1.85	<0.01	98	0.10	-0.49;0.69	0.74
Adjusted on season	157	0.86	0.35;1.37	<0.01	144	0.25	-0.24;0.74	0.31
Nulliparous women only	61	1.02	0.15;1.89	0.02	56	0.24	-0.50;0.98	0.52
Stations<10 km	103	0.56	-0.21;1.34	0.15	92	0.06	-0.51;0.63	0.83
<b>NO<sub>2</sub>, 90 days before the end of the index cycle<sup>c</sup></b>								
Continuous	134	0.48	-0.09;1.05	0.10	122	0.26	-0.28;0.80	0.34
<b>PM<sub>10</sub>, 30 days before the start of the index cycle</b>								
Continuous	156	1.15	-0.13;2.43	0.08	143	0.00	-1.27;1.27	1.00
<b>Sensitivity analyses restricted to</b>								
Regular cycles	123	1.11	-0.59;2.81	0.20	117	0.60	-0.66;1.87	0.35
No pregnancy in last 2 years	106	1.91	0.35;3.46	0.02	97	-0.56	-2.37;1.25	0.54
Adjusted on season	156	1.06	-0.16;2.28	0.09	143	-0.01	-1.29;1.27	0.98
Nulliparous women only	60	1.92	-0.30;4.13	0.09	55	-0.52	-2.39;1.35	0.57
Stations<10 km	88	1.07	-0.55;2.69	0.19	77	-0.54	-2.01;0.93	0.46
<b>PM<sub>10</sub>, 90 days before the end of the index cycle<sup>c</sup></b>								
Continuous	134	-0.65	-2.30;1.00	0.44	122	-0.14	-2.12;1.84	0.89

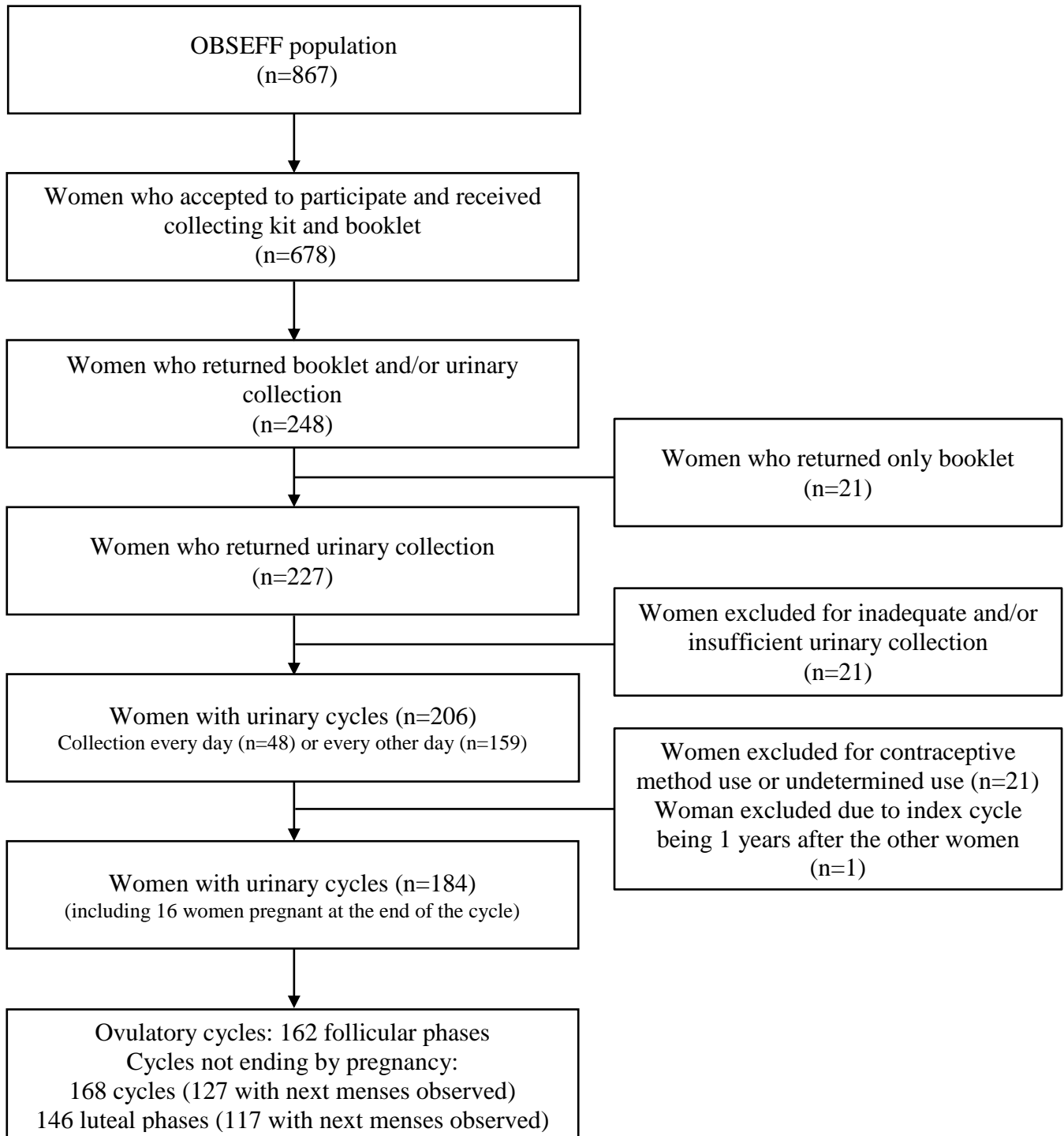
\*: Changes in mean duration associated with an increase by 10  $\mu\text{g}/\text{m}^3$  in air pollution level.

<sup>a</sup>: Linear regression adjusted on woman age (<30; 30-34; 35-39; 40-45 years), body mass index (<25,  $\geq$ 25  $\text{kg}/\text{m}^2$ , 3 missing values imputed in the lowest category), age at menarche (8-12; 12-18 years, 3 missing values imputed in the higher category), parity (nulliparous/parous), alcohol consumption in the week before inclusion (yes/no), caffeine consumption in the week before inclusion (yes/no), active smoking at inclusion (yes/no), passive smoking at inclusion (yes/no), education level (3 categories) and professional activity (yes/no)

<sup>b</sup>: Missing values for luteal phase (n=28) were imputed 1000 times using age and follicular length.

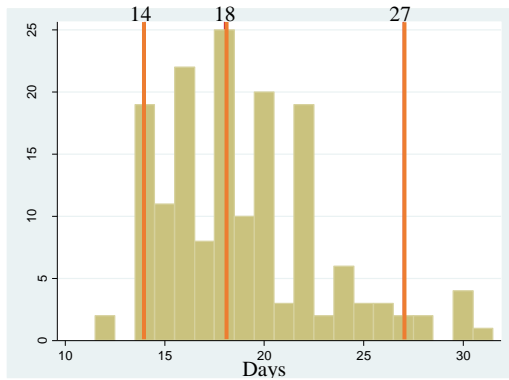
<sup>c</sup>: 90 days before the end of the index cycle (only available for women with observed menses at the end of the index cycle)

**Figure S III-1: Flow-chart**

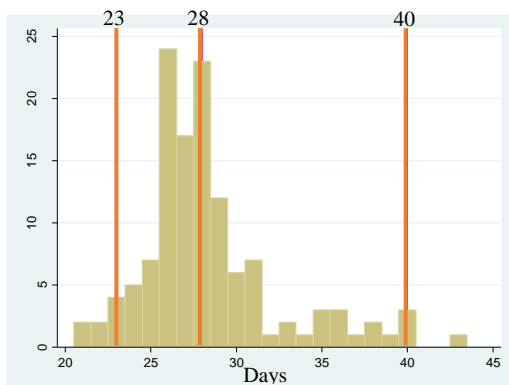


**Figure S III-2: Distribution of outcomes with 5<sup>th</sup>, 50<sup>th</sup> and 95<sup>th</sup> percentiles (orange lines)**

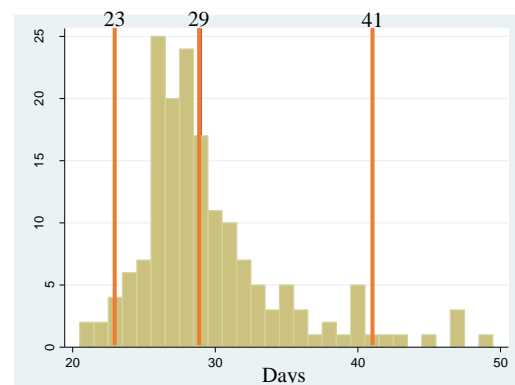
**A) Follicular phase length (n=162)**



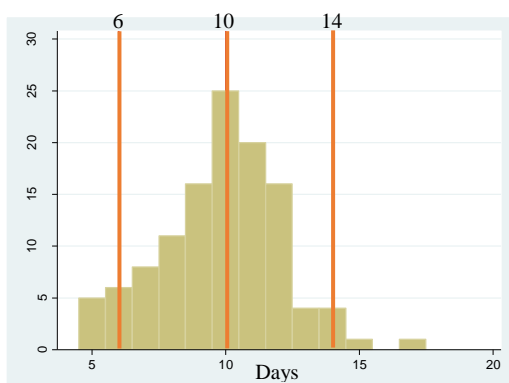
**B) Cycle length, not imputed (n=127)**



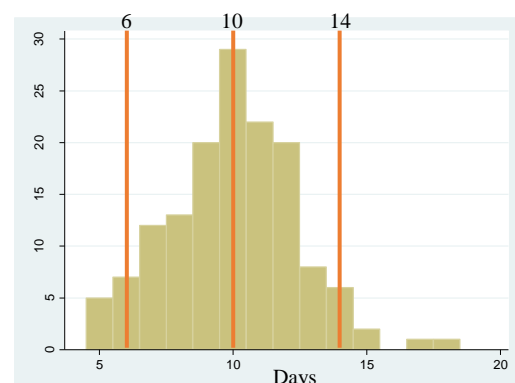
**C) Cycle length, 1 out 1000 imputations (n=168 women)**



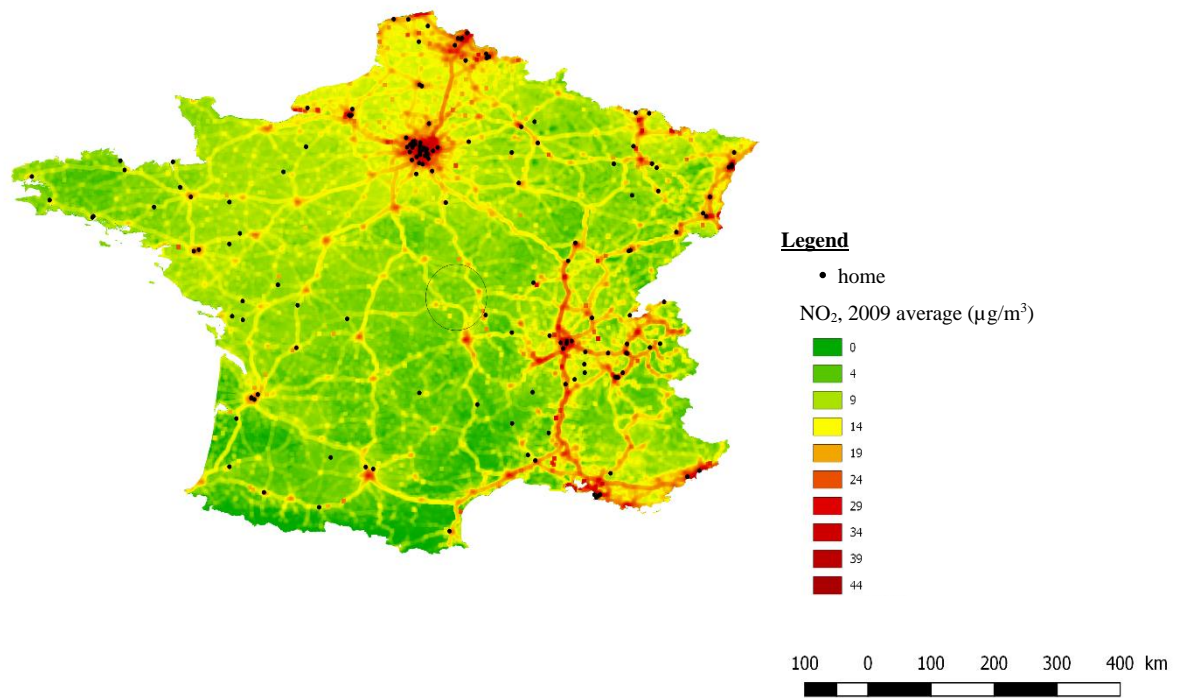
**D) Luteal phase length, not imputed (n=117)**



**E) Luteal phase length, 1 out of 1000 imputations (n=146 women)**



**Figure S III-3: Location of the home addresses of the study participants, and yearly NO<sub>2</sub> levels in France from the chemical-transport model during year 2009**





## Chapter IV: Around fertilization: Effects of Atmospheric pollution on couples' fecundity





## I. French summary

Introduction et objectif : Quelques études ont suggéré que la pollution atmosphérique pourrait influencer la spermatogénèse et la capacité biologique des couples à concevoir. Notre objectif était d'étudier l'impact que pourrait avoir l'exposition à la pollution atmosphérique à court terme sur la fertilité (capacité biologique à concevoir, « fecundity » en anglais).

Méthodes : Nous avons recruté dans l'OBServatoire de la Fertilité en France un échantillon aléatoire de couples n'utilisant pas de méthode contraceptive. Nous les avons dans un premier temps interrogés sur la durée depuis laquelle ils avaient arrêté d'utiliser une méthode de contraception (durée en cours sans contraception). L'exposition au dioxyde d'azote et aux particules au domicile de la femme pendant les 70 jours précédant l'arrêt de la contraception a été estimée par un modèle « Chemical-transport » combiné avec les données de la station de mesure de la qualité de l'air la plus proche du domicile. Un modèle de probabilité de défaillance en temps accéléré ajusté sur les principaux facteurs de confusion a été utilisé pour estimer l'association entre la pollution atmosphérique et la durée en cours sans contraception. Dans un second temps, les couples inclus dans l'approche des durées en cours et un échantillon aléatoire des couples non-éligibles ont été suivis pendant deux ans. Un modèle de Cox discret avec entrée différée a été utilisé pour étudier l'association entre les expositions à l'entrée dans le modèle et le délai nécessaire à concevoir.

Résultats : Dans l'approche des durées en cours, lorsque le niveau de PM<sub>10</sub> augmentait de 10 µg/m<sup>3</sup>, la durée en cours sans contraception médiane était multipliée par 1.29 (516 couples, Intervalle de confiance à 95% [0.97 ;1.70]). En prenant en compte les données de suivi, ni le dioxyde d'azote (477 couples) ni les particules (468 couples) n'étaient clairement associés au délai nécessaire à concevoir.

Discussion : Notre étude se basant sur deux designs en parallèle n'a pas observé d'association claire entre polluants atmosphériques et fertilité. Les précédentes études humaines et animales sur la fécondabilité (probabilité de grossesse dans le mois suivant l'arrêt de la contraception) ou sur des indicateurs de fertilité chez des souris ont été réalisées avec des niveaux d'exposition bien plus élevés que ceux observés dans notre étude, ce qui pourrait expliquer que nous ne retrouvons pas d'association claire dans notre population.

II. Article

**Do air pollutants have a short-term influence on couples' fecundity?  
An analysis relying on two study designs implemented in parallel.**

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*Short running title, not to exceed 50 characters and spaces*

Air pollution and couples' fecundity

*5–10 key words for indexing purposes (3/5-10)*

atmospheric pollutant, fecundity, time to pregnancy, current duration prevalent cohort

*Acknowledgments and funding*

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*Competing interests*

The funding sources had no role in the study design, collection, or interpretation of data, in the writing of the report, or in the decision to submit the paper for publication, with the exception of research staff of InVS who were implied in the development of parts of the study questionnaire not related to atmospheric pollution. The authors declare they have no competing financial interests.

*Abbreviations and definitions*

CDUI: Current Duration of Unprotected Intercourse, outcome considered in the current duration approach

CI: confidence interval

HR: Hazard Ratio

NO<sub>2</sub>: nitrogen dioxide

OBSEFF: Observatoire Epidémiologique de la Fertilité en France

PM<sub>10</sub>: particulate matter with aerodynamical diameter <10 µm

TR: Time Ratio

TTP: time to pregnancy, outcome considered in the prevalent cohort approach

Q0: recruitment interview occurring in 2007

Q1: first follow-up interview occurring in 2008

Q2: second follow-up interview occurring in 2009

## 1. Abstract

**Background:** Few studies have suggested a possible short-term impact of atmospheric pollutants on male fecundity parameters and on fecundity, a couple's ability to conceive a pregnancy.

**Objectives:** Our aim was to characterize the short-term influence of atmospheric pollutants on the fecundity of couples from the general population using two study designs.

**Methods:** As part of OBSEFF study, we recruited a random sample of couples not using regularly any contraceptive method. Couples were asked about the time elapsed since the start of the period of unprotected intercourse, which allowed defining the current duration of unprotected intercourse (CDUI). Couples were followed-up for two years, which corresponded to a prevalent cohort design, from which time to pregnancy was estimated. Levels of nitrogen dioxide (NO<sub>2</sub>) and of particulate matter with diameter below 10 µm (PM<sub>10</sub>) were averaged during the 70 days before the start of the period of unprotected intercourse (current duration analysis) and before the entry in the risk data set (prevalent cohort analysis) using data from nearest air quality monitoring station and a chemical-transport model. Adjusted analyses relied on an Accelerated Failure Time model (current duration analysis) or a survival model with delayed entry and right censoring (prevalent cohort analysis).

**Results:** In the current duration analysis, PM<sub>10</sub> levels (516 couples) and NO<sub>2</sub> levels (516 couples) were associated with a trend for increased duration of unprotected intercourse (for an increase of 10 µg/m<sup>3</sup>, median current duration multiplied by: 1.29 for PM<sub>10</sub>, 95% CI - confidence interval- 0.97;1.70, and 1.13 for NO<sub>2</sub>, 95% CI 1.05;1.22). In the prevalent cohort analysis, neither PM<sub>10</sub> levels (468 couples) nor NO<sub>2</sub> (477 couples) were associated with a decreased risk of pregnancy (Hazard Ratio of pregnancy for an increase of 10 µg/m<sup>3</sup>: 0.69 for PM<sub>10</sub>, 95%CI 0.43-1.12, and 0.90 for NO<sub>2</sub>, 95% CI 0.78-1.04).

**Discussion:** Our parallel analyses relying on the current duration and prevalent cohort designs that provide independent estimates were not in favor of a deleterious short-term effect of PM<sub>10</sub> or NO<sub>2</sub> on couple fecundity.

**Key words:** atmospheric pollutant, fecundity, time to pregnancy.

## 2. Introduction

Fecundity, the ability to conceive, is essential to species. In Humans, decrease in sperm quality has been reported in some area (Bonde et al., 2011; Pacey, 2013; Rolland et al., 2013) while incidence of testis cancer is increasing (Trabert et al., 2015).

Despite its importance, studying fecundity is challenging. Indeed, fecundity cannot be assessed directly, is optional, and couples “at risk” are difficult to identify. The historical approach to study fecundity is the retrospective time to pregnancy (TTP) design, in which parous women are asked about the time it took them to get pregnant. This approach suffers from the exclusion of couples not getting pregnant. Other designs allowing to include these couples exist, such as the current duration, the prevalent cohort and the incident cohort designs, in which couples are recruited during (current duration and prevalent cohort approaches) or before (incident cohort) the “at risk” period (Slama et al., 2014).

Atmospheric pollution has major impact on cardiovascular and respiratory mortality (Brunekreef and Holgate, 2002) and likely affects birthweight (Pedersen et al., 2013), but the literature about its effects on fecundity related outcomes is scarce (reviewed by Checa Vizcaíno et al., 2016 and Frutos et al., 2015). In animals, air pollution has been reported to decrease number of viable fetuses and increase implantation failure rates (Mohallem et al., 2005; Watanabe and Oonuki, 1999); follicle-stimulating hormone and luteinizing hormone were significantly decreased in young male rats exposed from birth to 3 months to diesel exhaust (Watanabe and Oonuki, 1999); longer oestrous cycles, reduced number of antral follicles and decreased fecundity have also been reported in mice (Veras et al., 2009). In humans, semen parameters, which are associated with a couple's fecundity (Bonde et al., 1998; Slama et al., 2002), might be influenced by atmospheric pollutants (reviewed by Lafuente et al., 2016). One pregnancy-based study in the Czech Republic first analyzed by Dejmek et al. (2000) and reanalyzed by Slama et al. (2013) reported a short term association between fine particulate matter (PM<sub>2.5</sub>), nitrogen dioxide (NO<sub>2</sub>) and some other pollutants and fecundability (the probability to conceive in the first month after stopping contraception) (Slama et al., 2013). In the US, a study observed that chronic exposures to particulate matter tended to be associated with incident infertility (Mahalingaiah et al., 2016) while another study in Barcelona, Spain, observed that higher levels of atmospheric pollution were associated with reduced fertility rates at the census tract level (Nieuwenhuijsen et al., 2014). No study focusing on the probability of pregnancy has been conducted in current population of Western countries and no study relied on designs allowing estimation of fecundability ratios considering couples remaining infertile.

It is unclear whether the association with fecundability observed in the Czech Republic in the 1990's would also exist in Western Europe at levels encountered today.

This study aimed to characterize short-term association between atmospheric pollution and fecundity, using two designs in parallel in a population including infertile couples: the current duration and the prevalent cohort approaches.



### 3. Methods

#### *Study population*

Our approach consisted of studying associations between a marker of fecundity and atmospheric pollution exposure using two designs, the current duration and the prevalent cohort designs, and relying on two overlapping populations. We relied on the OBSEFF population (Observatoire Epidémiologique de la Fertilité en France, see Slama et al., 2012 and Duron et al., 2013). A random sample of 943 couples was recruited by a phone survey among about 64,262 homes selected through random digit dialing in 2007 (questionnaire Q0). Couples living in urban areas of more than 100,000 inhabitants were over-sampled as at the time of the study, air pollution data were more easily available in urban areas. We used a weighting approach to correct this over-sampling, as well as to correct for differences (based on the distributions of age, marital life, number of children, age at the end of studies observed in the national census) between the interviewed women and the women from of the general population, as done in Slama et al (2012). Eligible women aged 18 to 44, living or having a regular relationship with a male partner, not using regularly any contraceptive method, who had sexual intercourse during the month before the interview Q0 and had not delivered during the three previous months were included at Q0. All couples eligible at Q0 as well as a random sample of 817 women aged 18 to 44 were followed-up one (Q1) and two years (Q2) after the first questionnaire. The current duration population is constituted of the 943 women eligible at Q0 while the prevalent cohort population is constituted of the 612 women eligible at Q0 that participated to the follow-up during one or two years, merged with the 98 women from the random sample of women not eligible at Q0 who had become eligible at the first follow-up Q1 and were followed-up one year later at Q2 (see Figure IV-1). As being actively trying to obtain a pregnancy was not an eligibility criterion, we did not exclude in the main analysis subfertile couples or couples considering themselves as infertile.

#### *Exposure assessment*

For women included in current duration design, we geocoded the home addresses at the time of the recruitment questionnaire (Q0). For women not eligible at Q0, we geocoded the follow-up addresses at the time of the first follow up questionnaire (Q1).

NO<sub>2</sub> and PM<sub>10</sub> levels at each home address were estimated from a chemical-transport exposure model developed by INERIS (French national institute for industrial environment and risks) and covering the whole country during a five year period (2009-2013) with high spatial

(1x1 km) and temporal (daily) resolutions (Benmerad et al., in press). As the exposure windows of interest occurred in 2004-2009, for each home address, pollutant and exposure window, we combined the exposure model estimates with measurements from the nearest background monitoring station at less than 100 km from home and operating during at least 75% of the exposure window (see supplemental material for details). Daily concentrations for all background monitoring stations in the country between January, 1<sup>st</sup> 2002 and July, 1<sup>st</sup> 2009 were retrieved from ADEME (French Environment & Energy Management Agency). In 2007, measurement methods changed in France to account for volatile fraction of PM<sub>10</sub> (Aymoz et al., 2008), thus to be able to compare levels within current duration and prevalent cohort population, only stations not measuring volatile fraction of PM<sub>10</sub> were selected.

### *Study outcomes*

In the current duration analysis, we are interested in the (unmeasured) time elapsed from the beginning of the current period of unprotected intercourse (corresponding to the cessation date of using a contraceptive method or, if no contraception was used, three month after the last pregnancy or the beginning of the actual relationship) to the pregnancy start (i.e. time to pregnancy: TTP), but as no follow-up is done, we can only observe the time from the beginning of the current period of unprotected intercourse until the recruitment interview (Q0), which is called the current duration of unprotected intercourse (CDUI). In the prevalent cohort, as the couples were followed-up during one or two years, we were able to see if the period of unprotected intercourse ended by a pregnancy or for any other reason (end of the relationship, resuming contraception) and we estimated the time to pregnancy. We defined two outcomes, the current duration of unprotected intercourse (CDUI), relevant for a current duration analysis, and the time to pregnancy (TTP), relevant for a prevalent cohort analysis.

### *Current duration design*

In the current duration design, couples are recruited while being “at risk” of pregnancy. We fitted an Accelerated Failure Time (AFT) model with CDUI as the outcome because it has been shown in this setting that the parameter of the AFT model associated with exposure is an estimation of the association between exposure and the total duration of unprotected intercourse if the system is in stationary state (Keiding et al., 2011; Yamaguchi, 2003). Results from AFT models are given as time ratios (TR). A TR of 2 corresponds to a doubling in the median time to the event of interest.

Analyses were restricted to couples with a CDUI shorter than 36 months and to couples not resorting to medical help for infertility trouble. This allows to censor these couples (Slama et al., 2006). Adjustments factors were defined at the start of the period of unprotected intercourse.

In the cross-sectional current duration approach, by design, the longer the CDUI is, the more ancient the start of the period of unprotected intercourse is. Thus, since exposure is defined with respect to the start of this period, when a decline in exposure exists, longer CDUI are associated with a higher exposure level at the beginning of the period of unprotected intercourse than shorter CDUI. This bias is similar to the one described by Weinberg and colleagues for the pregnancy-based approach (Weinberg et al., 1993) and it might biases the association between exposure and time to event towards higher time ratios with higher exposure. To quantify the part of the association due to this bias, we replicated the analysis by postponing all dates to a period in 2009-2010 in order to keep the seasonal and spatial patterns of exposure but removing the longer temporal trends. These exposures were estimated only from purely spatial model with the months and days of the exposure window postponed in 2009 (noted  $model_{dt_i,2009}(X_1, Y_1)_i$ , in the Appendix and corresponding to the exposure estimated at home address of woman  $i$  during the exposure window corresponding to the 70 days before the start of the period of unprotected intercourse of woman  $i$  postponed in 2009,).

#### *Prevalent cohort design*

In the prevalent cohort design, couples have been followed-up for up to two years and time to pregnancy (TTP) or to censoring events can be observed. A discrete-time survival model (logistic link) with delayed entry was used to estimate hazard ratios (HR) of pregnancy. Couples eligible in current duration design were included in the analysis at Q0 with delayed entry for the period of unprotected intercourse occurring between the beginning of the period of unprotected intercourse and Q0. For the couples not eligible at Q0 and becoming eligible before the first follow-up questionnaire Q1, the entry time corresponded to the time elapsed between the start of the period of unprotected intercourse and Q1. Couples were censored when resorting to medical help for infertility trouble, after 36 months of unprotected intercourse, when the women reached 45 years old, if the period of unprotected intercourse stopped for a reason other than achieving pregnancy or at the time of the last follow-up (Q2). All adjustment factors were defined with respect to the period just before the entry date in this approach.

### *Adjustment factors and exposure windows*

Adjustment factors were chosen a priori as those possibly affecting fecundity. Analyses were adjusted for woman age, active and passive smoking status, frequency of sexual intercourse, level of education and body mass index. For current duration analysis, they were defined at the start of the period of unprotected intercourse when available (woman age and active smoking) or at first interview (Q0) otherwise. For the prevalent cohort analysis, all adjustment factors were defined with respect to the period just before the entry date in the prevalent cohort (Q0 for women eligible at Q0 or Q1 for women not eligible at Q0).

Since the spermatogenesis lasts about 70 days (Heller and Clermont, 1964), we a priori chose an exposure window of 70 days before the start of the period at risk. For the current duration analysis, this corresponds to the 70 days preceding the start of the period of unprotected intercourse and for the prevalent cohort analysis to the 70 days preceding the entry date (Q0 or Q1).

### *Sensitivity analyses*

For both designs, sensitivity analyses were conducted by a) considering exposure windows of one month, three months and one year, b) restricting to couples for whom the distance between home and the closest station was below 10 km, c) adjusting for season and d) restricted to women who spend at least 75% of their time at home in the week before Q0 or Q1. Current duration analysis was e) restricted to women who did not move since the beginning of the period of unprotected intercourse (Table IV-3). Additionally, f) analyses were censored after a period of unprotected intercourse of 12 and 24 months instead of 36 months; analyses were restricted g) to pregnancy planners, h) nulliparous women, i) not menopausal women or j) period of unprotected intercourse longer than 1 month. Women with irregular use of contraception, who were included in the main analyses, were k) excluded and l) had their duration of unprotected intercourse arbitrarily halved. We also repeated current duration analysis m) without censoring for medical infertility treatment (i.e. not excluding) and n) excluding women that were pregnant at recruitment without knowing it. Prevalent cohort analysis was repeated o) including only women eligible in current duration analysis (i.e. eligible at Q0) (Table IV-4).

Statistical analyses were performed with Stata software (*Stata Statistical Software: Release 14.1*. College Station, TX: StataCorp LP.).

#### 4. Results

##### *Population.*

Among the 64,262 household randomly contacted, 15,810 women accepted to respond to the eligibility questionnaire; 943 (6%) were eligible and accepted to participate (Figure IV-2). CDUI could be defined for 867 of them (Slama et al., 2012). CDUI varied from a few days to 21 years (867 women with CDUI defined: median: 13.2 months, 5<sup>th</sup> percentile: 0.8 month, 95<sup>th</sup> percentile: 10 years). Among the group of 618 women who had a CDUI of less than 36 months, 544 had not resorted to medical help; corresponding to the population eligible in the current duration study. NO<sub>2</sub> exposure could be assessed for 521 women and PM<sub>10</sub> exposure for 519 women who lived at less than 100 km from a station in function with at least 75% of non-missing daily values. The localization of the home addresses for the women included in the current duration analysis is shown in Figure IV-3.

612 of the 943 women eligible in current duration study were followed up one or two years after recruitment Q0. 27 women were considered as non-eligible in the prevalent cohort because they gave inconsistent answers between recruitment Q0 and follow-up Q1, or indicated at follow-up Q1 that they were already pregnant at Q0. 240 of the 585 women included in both current duration and prevalent cohort studies became pregnant by the end of the follow-up. Among the 817 women not eligible at first questionnaire Q0 and were followed-up one (Q1) and two (Q2) year after, 98 were eligible (i.e. at risk of pregnancy) at Q1 and thus included the prevalent cohort study. 55 of these women became pregnant between Q1 and Q2 (Table IV-1). A total number of 683 women were included in the prevalent cohort study.

The characteristics of the eligible participants in current duration and prevalent cohort approaches are given in Table IV-1 while the detailed flow-chart is available in Figure IV-2.

##### *Associations between cofounders and time to pregnancy*

Woman age being below 25 years or higher than 35 years, lower frequency of sexual intercourse and body mass index superior to 25 kg/m<sup>2</sup> were associated with a trend for longer time to pregnancy in both designs while active smoking and body mass index inferior to 18.5 kg/m<sup>2</sup> were associated with increased probability of pregnancy in both designs. Higher levels of education were associated with higher probability of pregnancy in the prevalent cohort design but not in the current duration design. Passive smoking was associated with increased time to pregnancy in current duration design but with higher probability of pregnancy in the prevalent cohort design (Table IV-2).

*Air pollutants and time to pregnancy*

In the current duration design, the adjusted Time Ratio associated with a  $10 \mu\text{g}/\text{m}^3$  increase in  $\text{PM}_{10}$  levels in the 70 days before the start of the period of unprotected intercourse was 1.29 (95% CI [0.97;1.70],  $n=516$ ). When we used purely spatial exposures as a way to avoid the bias due to temporal (yearly) trends in exposure (i.e. exposures from the model with appropriate duration and period of the year, but postponed in 2009-2010), the association was not weakened: the adjusted time ratio increased to 2.19 (95% CI [1.76;2.72],  $n=516$ ). Respectively, the TR associated with a  $10 \mu\text{g}/\text{m}^3$  increase in  $\text{NO}_2$  levels was 1.13 (95% CI [1.05;1.22],  $n=516$ ) when the back-extrapolated exposures are used and 1.15 (95% CI [1.06;1.26],  $n=516$ ) with the purely spatial exposure. With the prevalent cohort design, the hazard ratio of pregnancy associated with a  $10 \mu\text{g}/\text{m}^3$  increase in  $\text{PM}_{10}$  levels in the 70 days before the entry was 0.69 (95% CI [0.47;1.12],  $n=468$ ) and 0.90 (95% CI [0.78;1.04],  $n=477$ ) with  $\text{NO}_2$ . When we restricted the distance between home address and the station used to backextrapolate to 10 km instead of 100 km, the association observed between atmospheric pollutants tended to strengthen in the current duration design ( $n=284$ , TR per  $10 \mu\text{g}/\text{m}^3$  increase in  $\text{PM}_{10}$  levels in the 70 days before the start of the period of unprotected intercourse: 1.83, 95% CI: 1.32;2.54, respectively  $n=335$ , TR:1.25, 95% CI 1.14;1.38 for  $\text{NO}_2$ ). In the prevalent cohort analysis, this strengthened association was observed for  $\text{PM}_{10}$  ( $n=258$ , HR of pregnancy per  $10 \mu\text{g}/\text{m}^3$  increase: 0.51, 95% CI: 0.23;1.13) but not for  $\text{NO}_2$  ( $n=300$ , HR of pregnancy per  $10 \mu\text{g}/\text{m}^3$  increase:0.91, 95% CI: 0.74;1.12). Detailed results with sensitivity analysis related to air pollution are available in Table IV-3.

*Sensitivity analyses*

Table IV-4 shows the results of the sensitivity analyses with restriction to various sub-populations. For both pollutants during the 70 days exposure window, the direction of the associations with probability of pregnancy remained constant in both designs. The sensitivity analysis that had most effects on the estimated TR and HR were the restriction to nulliparous women, which reduced strongly the number of subjects with only 42% of the women included in the current duration analysis being nulliparous. No clear association appeared in the prevalent cohort with exposure to  $\text{NO}_2$ , except to some extent when the women with irregular use of contraception were excluded ( $n=405$ , HR of pregnancy per  $10 \mu\text{g}/\text{m}^3$  increase in  $\text{NO}_2$ : 0.83, 95% CI: 0.70;0.98) but not when their time to pregnancy was halved ( $n=484$ , HR=0.91, 95% CI 0.79;1.06). This pattern was not observed with exposure to  $\text{PM}_{10}$  in prevalent cohort analysis

(n=397, HR of pregnancy per 10  $\mu\text{g}/\text{m}^3$  increase in  $\text{PM}_{10}$  when women with irregular contraception use were excluded: 0.78, 95% CI: 0.46;1.32. The corresponding HR was 0.71, 95% CI=0.43;1.17 when the total duration of unprotected intercourse of these women was halved). When an exposure window of 12 months was considered instead of 70 days, the HR for  $\text{NO}_2$  in the main analysis was 0.86 (n=477, 95% CI 0.73;1.02). This association remained stable through various sensitivity analysis and was not observed for  $\text{PM}_{10}$  (n=467, HR = 1.02, 95% CI: 0.60;1.74).

## 5. Discussion

This study is one of the first to characterize the impact of atmospheric pollutants on a marker of fecundity, and is the first to rely on the current duration and prevalent cohort designs. There was no clear evidence of a deleterious short-term association between atmospheric pollutants and fecundity.

### **Comparison with the literature**

The literature of air pollution effects on birth outcomes is very rich, but the one focusing on fecundity related outcomes is very limited, in particular in general population.

In Czech Republic, a study conducted in the years 1990's in Teplice city found an association between fecundability and PM<sub>2.5</sub> levels. The areas are not comparable in term of exposure as we focused on a whole country over a recent period (2004-2008) while Teplice was a highly industrialized city with high levels of PM<sub>2.5</sub> and SO<sub>2</sub> in 1993-1996. Median PM<sub>2.5</sub> level was 33 µg/m<sup>3</sup> in Teplice during 60 days, compared with 19 µg/m<sup>3</sup> for non-volatile PM<sub>10</sub> in our current duration study (70 days average before the start of the period of unprotected intercourse). This study did not considered the 12 months exposure windows. The other studies in general population context dealt with incident infertility (Mahalingaiah et al., 2016) or fertility rates (Koshal et al., 1980; Nieuwenhuijsen et al., 2014). Mahalingaiah et al observed a trend for an association between particulate matter and higher incident infertility with long term exposure windows (HR per 10 µg/m<sup>3</sup> increase in PM<sub>10</sub> from 1989 to estimated diagnosis date: 1.06; 95% CI [0.99;1.13]). Nieuwenhuijsen et al observed that annual coarse PM levels were associated with reduced fertility rates at census tract level in Barcelona in 2009. However, such an approach is probably limited by the strong influences of psychological, behavioral and social factors on fertility (Rossier and Bernardi, 2009), which are hard to control in an ecological setting, and which the focus on more biologically meaningful marker of fecundity such as TTP try to limit. In both cases, outcomes differed with our study.

### **Exposure assessment**

To estimate exposures, we combined a chemical-transport model covering the whole country to data from the nearest monitoring station. As the chemical-transport model was not available for the period when the couples began their period of unprotected intercourse (exposure window for the current duration analysis) or were interviewed (exposure window for



the prevalent cohort analysis), stations were needed to extrapolate the chemical-transport model back in time. Instead of assuming that spatial exposures from the chemical-transport model varied similarly over time than one representative background station in the area (Pedersen et al., 2013), we assumed that the spatial contrasts at the country scale were similar in 2009 than during the other years of the study period (2004-2008). This is a strong assumption but, at least for NO<sub>2</sub>, Lebret et al. (2000) observed that the geography of air pollution was relatively stable through the year. Using only estimates from station would have reduced the number of subjects included in current duration analysis as only 335 of them lived at less than 10 km from a station monitoring NO<sub>2</sub> (respectively 284 for PM<sub>10</sub>) and in prevalent cohort analysis (304 women for NO<sub>2</sub> and 261 for PM<sub>10</sub>). As stations are mostly located in urban area, most women from rural areas would be excluded from such an analysis.

As it is often the case in air pollution epidemiology, our exposure estimates did not take space-time activity into account. We collected some information on space-time activity by asking to women how many hours they left from home each day in the week before Q0 for women eligible in current duration design and before Q1 otherwise. 60% of the women participating in the current duration analysis and 54% of those participating in the prevalent cohort analysis declared that they spent at least 75% of the week at home. When the analyses were restricted to those women, point estimates were moved away from the null for NO<sub>2</sub> but not PM<sub>10</sub>.

### **Study design**

Since the current duration analysis relies on the time before the recruitment interview, while the outcome of the prevalent cohort analysis is the time until a potential pregnancy, these two approaches can be seen as being independent analyses although they largely rely on the same population. The current duration design has so far very little been used and mostly for descriptive purposes, in France for the OBSEFF study (Slama et al., 2012) and its feasibility study (Slama et al., 2006), in the USA in the 2002 National Survey of Family Growth (Louis et al., 2013; Thoma et al., 2013) and in a cross sectional study of women in Denmark, Germany and Italy (Keiding et al., 2002). To our knowledge, this study is the first relying on the current duration design to identify risk factors of altered fecundity. The prevalent cohort design has been used in the Danish *Snart-Gravid* study (Wildenschild et al., 2015; Wise et al., 2010).

The direction of the associations with atmospheric pollution or adjustment factors was consistent in our analysis for current duration and prevalent cohort designs with the exception

of passive smoking and education level. Censoring is done in the current duration design by excluding couples while in the prevalent cohort design classical right-censoring as in an incident cohort can be done. The choice of the cut-off for censoring has different impact depending on the design used: censoring at 6 years instead of 3 years modified the relation between age at the start of the period of unprotected intercourse and CDUI: women aged 40-44 seemed to have an increased fecundity compared to the reference group (25-29) (TR: 0.93, 95% CI: 0.55;1.58 when the analysis is censored after a period of unprotected intercourse of 6 years to compare with a TR of 1.25, 95% CI: 0.71;2.18 when the analysis is censored after a period of unprotected intercourse of 3 years) while this effect was not present in the prevalent cohort analysis (HR: 0.31, CI 0.15;0.68 when the analysis is censored after a period of unprotected intercourse of 6 years to compare with a HR of 0.32, 95% CI: 0.15;0.72 when the analysis is censored after a period of unprotected intercourse of 3 years).

### **Biases**

Time to pregnancy studies are known to be potentially impacted by several biases (Slama et al., 2006, 2014; Weinberg et al., 1993, 1994).

Time trends in exposure can bias the results (Weinberg et al., 1993; Sallmén et al., 2000) of such analyses. In our study, atmospheric pollutants levels decreased gradually during the study period, which is an issue for the current duration (but not the prevalent cohort) approach. Indeed, the exposure window used in the current duration approach took place before the beginning of the period of unprotected intercourse: couples with longer CDUI will have higher estimated exposure levels than couples with shorter CDUI. We aimed to correct for this bias by relying on a purely spatial model exposure, which can be seen as an instrumental variable. When we replicated the main analysis with the purely spatial exposures, the estimated TR of 1.13 (95% CI: 1.05;1.22) per each increase by  $10 \mu\text{g}/\text{m}^3$  in  $\text{NO}_2$  average during the 70 days before the start of the period was modified to 1.15 (95% CI: 1.06;1.26). Similarly, for  $\text{PM}_{10}$ , the estimated TR of 1.29 (95% CI: 0.97;1.70) per each increase by  $10 \mu\text{g}/\text{m}^3$  during the 70 days before the start of the period was modified to 2.19 (95% CI 1.76;2.72). Although the results of the analysis with purely spatial exposure were not in favor of the main current duration analysis results being biased by the presence of time trends in exposures, the results of the current duration analysis should be taken with caution as it is possible that using the purely spatial exposure may not be enough to remove the bias and as the prevalent cohort -not affected by this bias as the exposure window is related to the entry in the risk data set- approach results are not clearly in favor of an association between air pollution levels and probability of pregnancy.

The only association observed using the prevalent cohort design was for NO<sub>2</sub> when the 12 month exposure windows was used. However, if this association was also observed with the current duration design when the distance between home and station is restricted to 10 km (n=332, TR = 1.21, 95% CI = 1.07;1.37), when purely spatial exposures were used to remove the temporal trend due to decreasing air pollutants levels in the study period, the strength of the association was decreased (n=332, TR = 1.11, 95% CI: 0.95;1.30).

The AFT analysis of current duration of unprotected intercourse data assumes a lack of trends in initiating times. This assumption might not be true as when ineligible women were followed up during one year after recruitment, among the 447 women that began a period of unprotected intercourse in the year following the recruitment date Q0, 28% did it in January-March, 37% in April-June, 17% in July-September and 18% in October-December (respectively 30%, 36%, 18%, and 16% for the 368 pregnancy planners). We were not able to identify all the couples with an initiated period of unprotected intercourse ending by the couple resuming contraception, but we believe it is unlikely that they would have modify the distribution of the period of unprotected intercourse onsets to be more stable. Since the prevalent cohort design does not rely on a stationarity assumption and since the directions of the associations were similar for both designs, the impact of this bias may have only affected the size of the estimates of the current duration analysis.

Another difficulty of time to pregnancy studies is the exclusion of unplanned or mistimed pregnancies and of non-pregnancy planner. It is not possible to include pregnancies due to birth control failures in either current duration or prevalent designs. Our questionnaires allowed to identify couples actively trying to conceive and also couples at risk of pregnancy but not considering themselves as being trying to have a child. Our main analyses included couples not actively trying, but the main results remained stable when the analyses were restricted to pregnancy planners.

Recognition bias and recall bias can also be an issue. Questions about previous pregnancies were asked at three different times in each questionnaire, allowing to reconstruct a complete pregnancy history. Using only one question would have made us miss some miscarriages or other pregnancies not ending with a live birth. Amongst women included in the current duration study and followed-up for the prevalent cohort, 14 (2%) declared at the follow-up that they were pregnant at the time of the recruitment questionnaire (Q0). Excluding these women did not change the results. A similar number of women (n=13, 2%) declared at first follow-up (Q1) that they were using contraception at the time of the recruitment questionnaire

(Q0). These 27 women were excluded from the prevalent cohort approach but not from the main current duration analyses.

Couples may modify their behavior (for example stop smoking) if they do not succeed to have a child. Whenever possible, to avoid the behavior modification bias, adjustment factors were considered at the beginning of the period of unprotected intercourse for the current duration analysis. Some variables were only available at the recruitment interview Q0 (body mass index, exposure to passive smoking) which may have entailed bias if the behavior has been modified since the start of the period of unprotected intercourse. Since in the prevalent cohort analysis the couples entered the risk set with delayed entry, the adjustments factors were considered at entry (recruitment Q0 for women included in current duration analysis, first follow-up Q1 otherwise) and thus their assessment is less likely to suffer from recall bias.

## 6. Conclusion

Our study based on two study designs was not clearly in favor of a short-term adverse effect of atmospheric pollution on couple fecundity.

Very few studies have considered the association between atmospheric pollution and fecundity or (in)fertility. One strength of our study is that we used an exposure model with daily and 1 x 1 km resolutions back-extrapolated using data from stations. We were able to consider a large set of individual confounders and we considered many biases known to happen in the context of time to pregnancy studies (in particular bias due to time-trends in exposure) and were able to conduct in parallel analyses using two study designs that include infertile couples in the same population.

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## 8. Tables and figures

**Table IV-1: Characteristics of the population included in current duration or prevalent cohort studies with defined CDUI or time to pregnancy. Note that 585 subjects of the prevalent cohort analysis are also included in the current duration analysis.**

	Current duration (n=867)				Prevalent cohort, participation in current duration (n=585)				Prevalent cohort, not eligible in current duration (n=98)			
	N	%	%*	Median CDUI [SD]	N	%	%*	Median time to event or censor [SD]	N	%	%*	Median time to event or censor [SD]
<b>Women characteristics</b>												
<b>Duration of unprotected intercourse</b>												
<3 months	182	21	20		47	8	8		10	1	1	
3 months-1 year	231	27	26		121	20	18		44	5	5	
1-2 years	130	15	16		102	17	17		36	4	4	
2-3 years	75	9	9		81	13	13		8	1	1	
3-6 years	133	15	15		131	21	23		0	0	0	
≥6 years	116	13	14		103	17	18		0	0	0	
<b>Infertility treatment</b>												
No	708	82	83	9 [37]	464	80	80	21 [47]	83	85	83	10 [7]
yes	159	18	17	40 [54]	121	21	20	37 [29]	15	15	17	15 [8]
<b>Age of the woman<sup>(a)</sup></b>												
<25	101	12	16	17 [61]	32	5	8	20 [19]	6	6	16	7 [6]
25-29	263	30	33	12 [46]	130	22	26	13 [22]	38	39	35	8 [6]
30-34	293	34	30	13 [39]	178	30	32	19 [30]	28	29	25	10 [6]
35-39	175	20	18	14 [27]	152	26	22	41 [39]	18	18	15	18 [7]
40-44	35	4	3	9 [14]	93	16	13	90 [61]	8	8	10	22 [8]
<b>Education level<sup>(b)</sup></b>												
< baccalaureate	212	24	41	16 [47]	127	22	37	40 [48]	8	8	20	16 [10]
baccalaureate	350	40	37	15 [41]	225	38	36	26 [41]	41	42	37	11 [6]
> baccalaureate	305	35	22	10 [40]	233	40	26	22 [44]	43	44	35	10 [7]
missing									6	6	9	16 [7]
<b>Frequency of sexual intercourse<sup>(b)</sup></b>												
1-3 per month	165	19	18	18 [50]	113	19	19	34 [52]	14	14	15	13 [7]
1-2 per week	418	48	46	13 [42]	299	51	50	27 [45]	53	54	50	11 [7]
≥3 per week	269	31	34	11 [38]	167	29	30	20 [34]	25	26	27	8 [7]
missing	15	2	2	15 [31]	6	1	2	57 [36]	6	6	9	16 [7]
<b>Active smoking<sup>(a)</sup></b>												
No	580	67	61	13 [41]	411	70	66	26 [42]	67	68	56	11 [7]
Yes	279	32	38	13 [46]	174	30	34	28 [48]	25	26	35	9 [7]
Missing	8	1	1	20 [22]					6	6	9	16 [7]
<b>Passive smoking<sup>(b)</sup></b>												
No	513	59	51	13 [44]	365	62	55	27 [46]	62	63	59	10 [7]
Yes	354	41	49	13 [40]	220	38	45	27 [40]	30	31	32	10 [7]
Missing									6	6	9	16 [7]
<b>Body Mass Index (μg/m<sup>3</sup>)<sup>(b)</sup></b>												
<18.5	78	9	11	8 [43]	46	8	9	14 [36]	6	6	8	7 [3]
18.5-24.9	543	63	57	13 [44]	375	64	61	28 [47]	71	72	71	11 [7]
≥25	233	27	30	15 [38]	159	27	30	28 [39]	15	15	13	9 [8]
missing	13	1	2	17 [38]	5	1	1	18 [46]	6	6	9	16 [8]



Effects of atmospheric pollution on couples' fecundity

	Current duration (n=867)				Prevalent cohort, participation in current duration (n=585)				Prevalent cohort, not eligible in current duration (n=98)			
	N	%	%*	Median CDUI [SD]	N	%	%*	Median time to event or censor [SD]	N	%	%*	Median time to event or censor [SD]
Women characteristics												
End of the period of unprotected intercourse												
Pregnancy	N.A.				240	41	38	9 [9]	55	56	58	8 [5]
Censored due to end of follow-up	N.A.				51	9	9	27 [7]	29	30	29	20 [5]
Censored for other reason	N.A.				294	50	53	55 [48]	14	14	13	10 [5]
Air pollution (mean [SE])												
NO <sub>2</sub> <sup>(a)</sup>	732	18.4 [0.5]		9 [18]	580	21.2 [0.5]		27 [43]	97	29.4 [1.8]		11 [7]
PM <sub>10</sub> <sup>(a)</sup>	731	18.7 [0.2]		9 [18]	578	19.0 [0.1]		27 [43]	89	19.8 [0.4]		11 [7]

\* Percentages were corrected for possible selection bias and over-representation of urban compared with rural areas.

N.A.: not applicable

CDUI: current duration of unprotected intercourse

(a) At the start of the current duration of unprotected intercourse for current duration approach, and for prevalent cohort approach at recruitment (Q0) for women eligible in current duration approach or at first follow-up interview (Q1) otherwise.

(b) at recruitment interview (Q0) for women eligible in current duration study, at first follow-up (Q1) for women not eligible in the current duration study

**Table IV-2 Association between adjustment factors and time to pregnancy. Current duration and prevalent cohort approach.**

	Current duration n=528			Prevalent cohort n=481		
	N	TR	95% CI	N	HR	95% CI
Age <sup>a</sup>						
<25	56	1.32	0.98;1.77	32	0.97	0.53;1.77
25-29	166	1.00	reference	141	1.00	reference
30-34	168	0.85	0.67;1.09	166	1.18	0.85;1.64
35-39	113	1.32	0.99;1.77	106	0.57	0.37;0.86
40-44	25	1.25	0.71;2.18	36	0.32	0.15;0.72
Education <sup>b</sup>						
<baccalaureate	121	1.00	reference	77	1.00	reference
Baccalaureate	206	1.00	0.78;1.28	200	2.18	1.34;3.54
> baccalaureate	201	1.00	0.77;1.30	204	2.16	1.31;3.54
Frequency of sexual intercourse <sup>b</sup>						
1-3 per month	97	1.31	0.98;1.74	81	0.42	0.26;0.68
1-2 per week	255	1.24	0.99;1.55	255	0.81	0.60;1.10
>=3 per week	176	1.00	reference	145	1.00	reference
Active smoker <sup>a</sup>						
No	359	1.00	reference	343	1.00	reference
Yes	169	0.91	0.72;1.17	138	1.09	0.79;1.52
Passive smoking <sup>b</sup>						
No	304	1.00	reference	298	1.00	reference
Yes	224	1.28	1.03;1.59	183	1.03	0.75;1.42
Body mass index <sup>b</sup> (kg/m <sup>2</sup> )						
<18.5	55	0.84	0.60;1.17	42	1.30	0.80;2.11
18.5-25	331	1.00	reference	317	1.00	reference
>25	142	1.05	0.83;1.32	122	0.73	0.52;1.04

(a) At the start of the current duration of unprotected intercourse for current duration approach, and for prevalent cohort approach at recruitment (Q0) for women eligible in current duration approach or at first follow-up interview (Q1) otherwise.

(b) at recruitment interview (Q0) for women eligible in current duration study, at first follow-up (Q1) for women not eligible in the current duration study

TR: Time ratio. A time ratio higher than 1 indicates a reduced fecundity level

HR: Hazard ratio. A hazard ratio lower than 1 indicates a reduced fecundity level

Current duration: Accelerated failure time model, adjusted for woman age and active smoking at the beginning of the period of unprotected intercourse and education level, frequency of sexual intercourse, passive smoking and body mass index at recruitment interview (Q0).

Prevalent cohort: Discrete time survival model adjusted for woman age, active smoking, education level, frequency of sexual intercourse, passive smoking and body mass index at recruitment interview (Q0) for women eligible in current duration study, at first follow-up (Q1) for women not eligible in the current duration study.

All analyses are censored after 36 months.

**Table IV-3: Atmospheric pollutants and time to pregnancy. Current duration and prevalent cohort approach.**

Analysis and exposures windows*	NO <sub>2</sub>								PM <sub>10</sub>							
	Current duration approach				Prevalent cohort approach				Current duration approach				Prevalent cohort approach			
	N	TR	p-value	95% CI	N	HR	p-value	95% CI	N	TR	p-value	95% CI	N	HR	p-value	95% CI
Home-station distance restricted to 100 km:																
1 month	514	1.07	0.07	0.99;1.15	475	0.90	0.13	0.79;1.03	516	1.21	0.11	0.96;1.52	468	0.83	0.40	0.53;1.29
70 days	516	1.13	<0.01	1.05;1.22	477	0.90	0.14	0.78;1.04	516	1.29	0.08	0.97;1.70	468	0.69	0.13	0.43;1.12
3 months	515	1.15	<0.01	1.06;1.24	476	0.90	0.16	0.78;1.04	515	1.32	0.05	1.00;1.74	468	0.79	0.34	0.48;1.28
1 year	516	1.06	0.24	0.96;1.16	477	0.86	0.08	0.73;1.02	506	1.14	0.44	0.82;1.60	467	1.02	0.94	0.60;1.74
Home-station distance restricted to 10 km:																
1 month	331	1.16	<0.01	1.06;1.26	299	0.92	0.43	0.76;1.12	284	1.44	<0.01	1.12;1.86	258	0.80	0.48	0.43;1.48
70 days	335	1.25	<0.01	1.14;1.38	300	0.91	0.39	0.74;1.12	284	1.83	<0.01	1.32;2.54	258	0.51	0.10	0.23;1.13
3 months	335	1.30	<0.01	1.18;1.44	300	0.92	0.40	0.74;1.13	285	1.95	<0.01	1.40;2.71	258	0.51	0.12	0.22;1.19
1 year	332	1.21	<0.01	1.07;1.37	300	0.85	0.19	0.66;1.09	281	1.36	0.13	0.91;2.04	258	0.70	0.35	0.33;1.49
Purely spatial exposure model																
1 month	514	1.10	0.02	1.02;1.19	475	0.95	0.48	0.81;1.10	516	1.75	<0.01	1.49;2.07	468	0.83	0.27	0.60;1.15
70 days	516	1.15	<0.01	1.06;1.26	477	0.93	0.38	0.79;1.09	516	2.19	<0.01	1.76;2.72	468	0.77	0.17	0.53;1.12
3 months	515	1.15	<0.01	1.06;1.26	476	0.92	0.36	0.78;1.09	515	2.31	<0.01	1.81;2.96	468	0.76	0.18	0.51;1.13
1 year	516	0.98	0.71	0.88;1.09	477	0.93	0.45	0.78;1.12	506	1.19	0.39	0.80;1.79	467	0.68	0.24	0.36;1.29
Additional adjustment on season*																
1 month	514	1.02	0.68	0.93;1.12	475	0.91	0.16	0.80;1.04	516	1.25	0.08	0.97;1.60	468	0.82	0.39	0.53;1.28
70 days	516	1.04	0.49	0.94;1.14	477	0.91	0.18	0.78;1.05	516	1.39	0.02	1.05;1.85	468	0.71	0.16	0.43;1.15
3 months	515	1.04	0.47	0.94;1.14	476	0.91	0.23	0.78;1.06	515	1.35	0.04	1.02;1.79	468	0.81	0.40	0.49;1.33
1 year	516	1.06	0.28	0.96;1.17	477	0.86	0.08	0.72;1.02	506	1.30	0.14	0.92;1.83	467	0.96	0.87	0.56;1.63
Women who spent 75% of a week at home only**																
1 month	282	1.10	0.09	0.99;1.22	244	0.84	0.07	0.70;1.01	284	1.09	0.58	0.81;1.45	239	0.73	0.29	0.41;1.31
70 days	283	1.17	0.01	1.04;1.31	245	0.85	0.12	0.69;1.05	284	1.21	0.30	0.85;1.72	239	0.79	0.48	0.42;1.50
3 months	282	1.19	<0.01	1.06;1.34	244	0.85	0.13	0.68;1.05	283	1.21	0.29	0.85;1.73	239	1.02	0.94	0.54;1.96
1 year	283	1.10	0.13	0.97;1.26	245	0.77	0.03	0.60;0.98	278	1.16	0.49	0.75;1.79	239	1.20	0.64	0.56;2.54
Same address since contraception stop																
1 month	447	1.03	0.52	0.94;1.13	NA				447	1.15	0.26	0.90;1.48	NA			
70 days	447	1.09	0.06	1.00;1.20	NA				447	1.39	0.04	1.01;1.90	NA			
3 months	447	1.11	0.03	1.01;1.22	NA				447	1.38	0.04	1.01;1.88	NA			
1 year	447	1.01	0.83	0.91;1.13	NA				439	1.31	0.17	0.89;1.93	NA			

TR: Time ratio. A time ratio higher than 1 indicates a reduced fecundity level  
 TRs and HRs are reported for an increase by 10 µg/m<sup>3</sup> in air pollution

HR: Hazard ratio. A hazard ratio lower than 1 indicates a reduced fecundity level  
 All analyses are censored after 36 months.

NA: not applicable

\* At the start of the current duration of unprotected intercourse for current duration approach, and for prevalent cohort approach at recruitment (Q0) for women eligible in current duration approach or at first follow-up interview (Q1) otherwise.

\*\* Estimated on the week before Q0 for women eligible in current duration study, on the week before Q1 otherwise

Current duration: Accelerated failure time model, adjusted for woman age and active smoking at the beginning of the period of unprotected intercourse and education level, frequency of sexual intercourse, passive smoking and body mass index at recruitment interview (Q0).

Prevalent cohort: Discrete time survival model adjusted for woman age, active smoking, education level, frequency of sexual intercourse, passive smoking and body mass index at recruitment interview (Q0) for women eligible in current duration study, at first follow-up (Q1) for women not eligible in the current duration study.

**Table IV-4: Atmospheric pollutants and time to pregnancy. Current duration and prevalent cohort approach, sensitivity analyses**

	NO <sub>2</sub>								PM <sub>10</sub>							
	Current duration approach				Prevalent cohort approach				Current duration approach				Prevalent cohort approach			
	N	TR	p-value	95% CI	N	HR	p-value	95% CI	N	TR	p-value	95% CI	N	HR	p-value	95% CI
<b>Main analysis</b>																
Air pollution, 70 days**	516	1.13	<0.01	1.05;1.22	477	0.90	0.14	0.78;1.04	516	1.29	0.08	0.97;1.70	468	0.69	0.13	0.43;1.12
<b>Sensitivity analysis, 70 days exposure window**</b>																
Censor																
12 months	375	1.14	<0.01	1.04;1.24	343	0.89	0.23	0.74;1.07	374	1.42	<0.01	1.12;1.80	335	0.92	0.78	0.50;1.67
24 months	472	1.13	<0.01	1.05;1.21	433	0.92	0.29	0.79;1.07	472	1.58	<0.01	1.22;2.03	424	0.81	0.42	0.49;1.35
Treatment not censored	584	1.14	<0.01	1.06;1.22					584	1.29	0.04	1.01;1.65				
Pregnancy planners only	449	1.13	<0.01	1.04;1.22	427	0.91	0.22	0.79;1.06	450	1.40	0.03	1.03;1.91	417	0.78	0.33	0.48;1.28
Nulliparous women only*	217	1.03	0.55	0.93;1.15	206	1.00	0.99	0.83;1.20	218	1.59	0.03	1.04;2.45	202	0.80	0.52	0.41;1.57
Menopausal women excluded	512	1.13	<0.01	1.05;1.22	474	0.90	0.14	0.78;1.03	512	1.29	0.08	0.97;1.70	465	0.66	0.09	0.40;1.07
Couples with irregular use of contraception are excluded	436	1.15	<0.01	1.06;1.25	405	0.83	0.03	0.70;0.98	435	1.24	0.17	0.91;1.69	397	0.78	0.35	0.46;1.32
have their duration of unprotected intercourse halved	531	1.14	<0.01	1.05;1.23	484	0.91	0.22	0.79;1.06	531	1.27	0.09	0.96;1.67	475	0.71	0.18	0.43;1.17
Duration of unprotected intercourse of less than one month excluded	460	1.14	<0.01	1.06;1.22	472	0.92	0.23	0.79;1.06	460	1.27	0.06	0.99;1.63	463	0.69	0.14	0.42;1.13
Women not eligible in current duration approach excluded					396	0.87	0.10	0.74;1.03					395	0.80	0.42	0.47;1.38
Excluding from current duration analysis women declaring at follow-up that they were pregnant at recruitment	511	1.13	<0.01	1.05;1.22					511	1.28	0.08	0.97;1.70				

TRs and HRs are reported for an increase by 10 µg/m<sup>3</sup> in air pollution

TR: Time ratio. A time ratio higher than 1 indicates a reduced fecundity level

HR: Hazard ratio. A hazard ratio lower than 1 indicates a reduced fecundity level

All analyses are censored after 36 months

Current duration: Accelerated failure time model, adjusted for woman age and active smoking at the beginning of the period of unprotected intercourse and education level, frequency of sexual intercourse, passive smoking and body mass index at recruitment interview (Q0).

Prevalent cohort: Discrete time survival model adjusted for woman age, active smoking, education level, frequency of sexual intercourse, passive smoking and body mass index at recruitment interview (Q0) for women eligible in current duration study, at first follow-up (Q1) for women not eligible in the current duration study.

\*Analyses restricted to nulliparous women were not weighted in the prevalent cohort approach due to convergence issues.

\*\* At the start of the current duration of unprotected intercourse for current duration approach, and for prevalent cohort approach at recruitment (Q0) for women eligible in current duration approach or at first follow-up interview (Q1) otherwise.

**Figure IV-1: Study design, example for five women (A to E)**

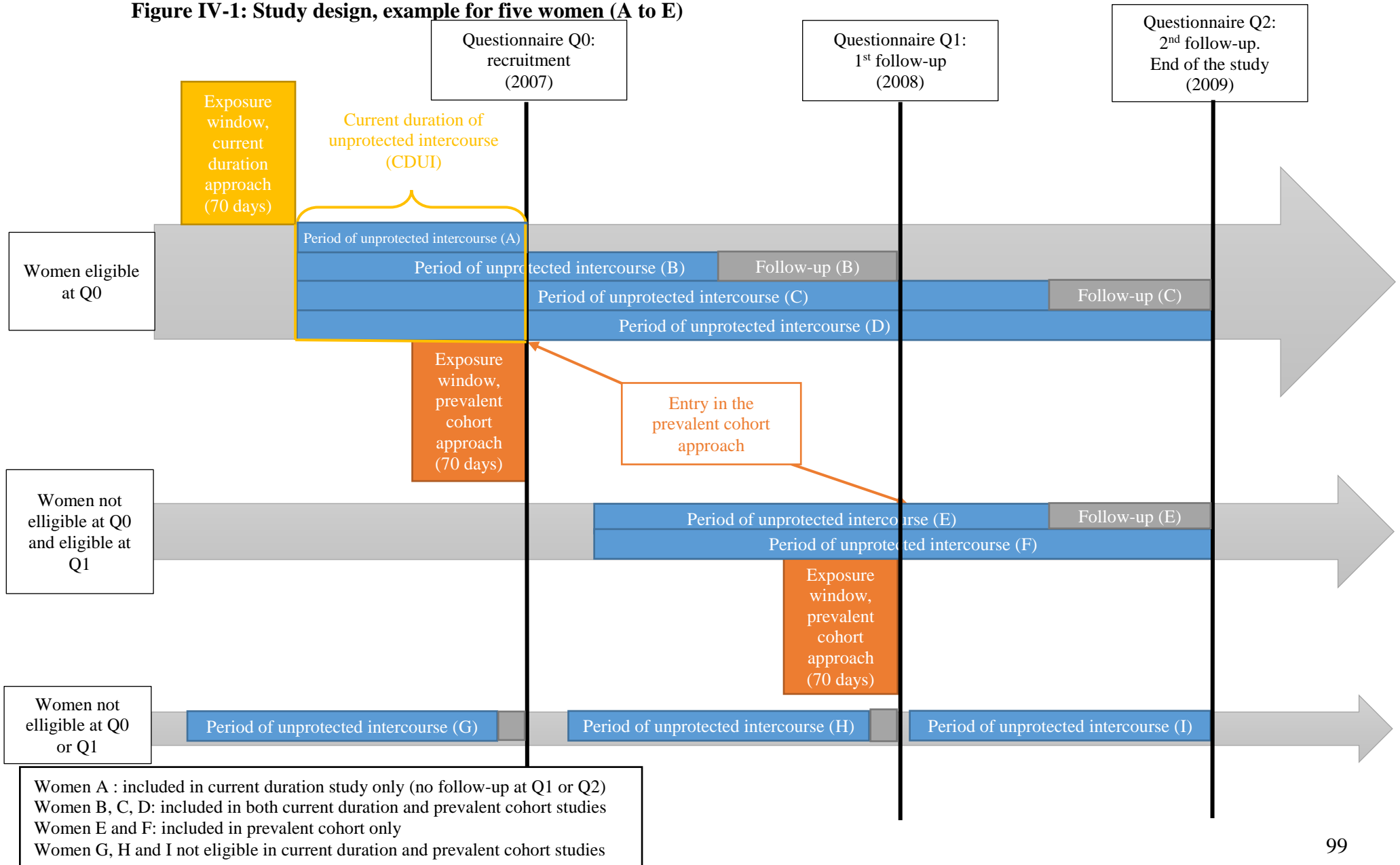
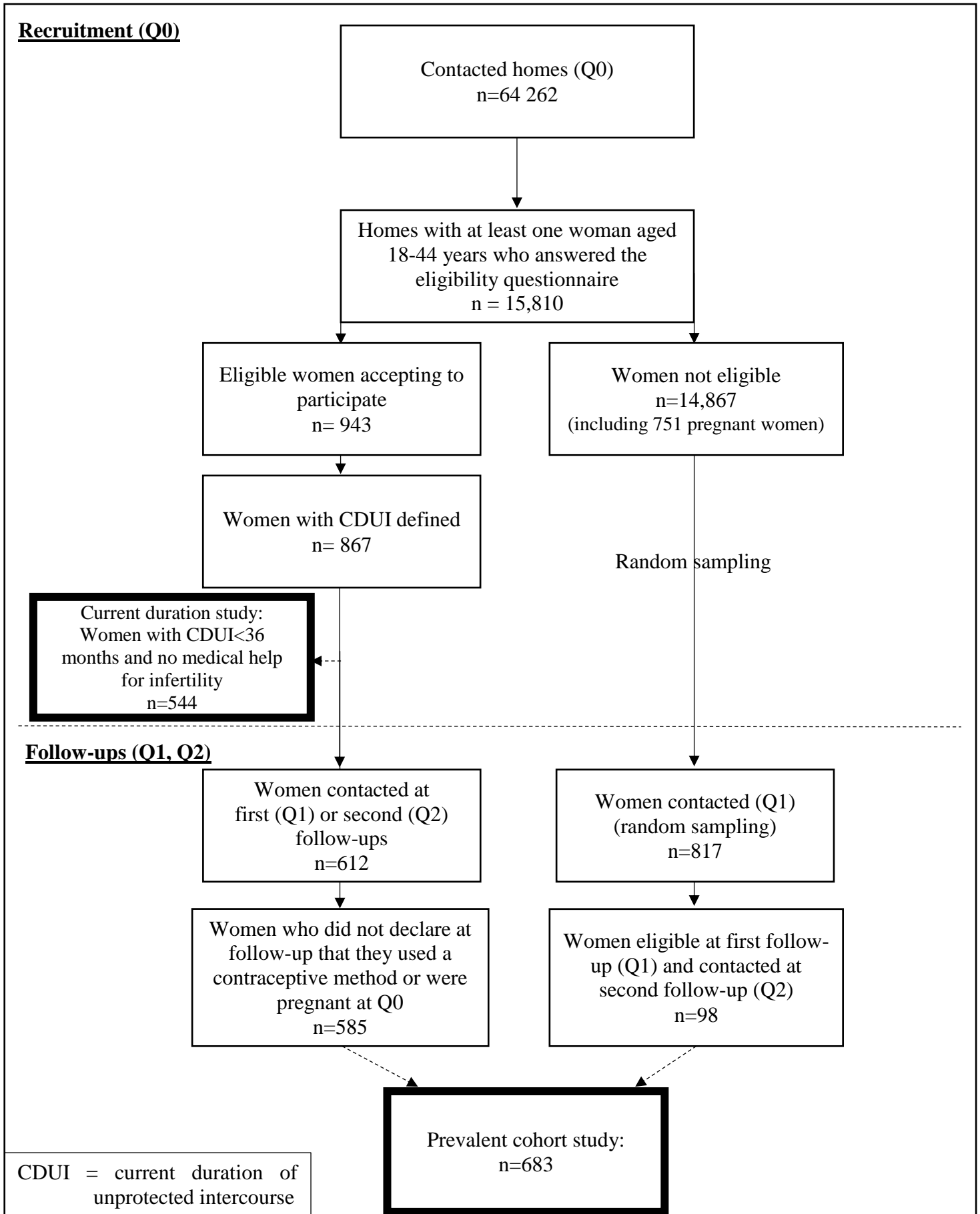
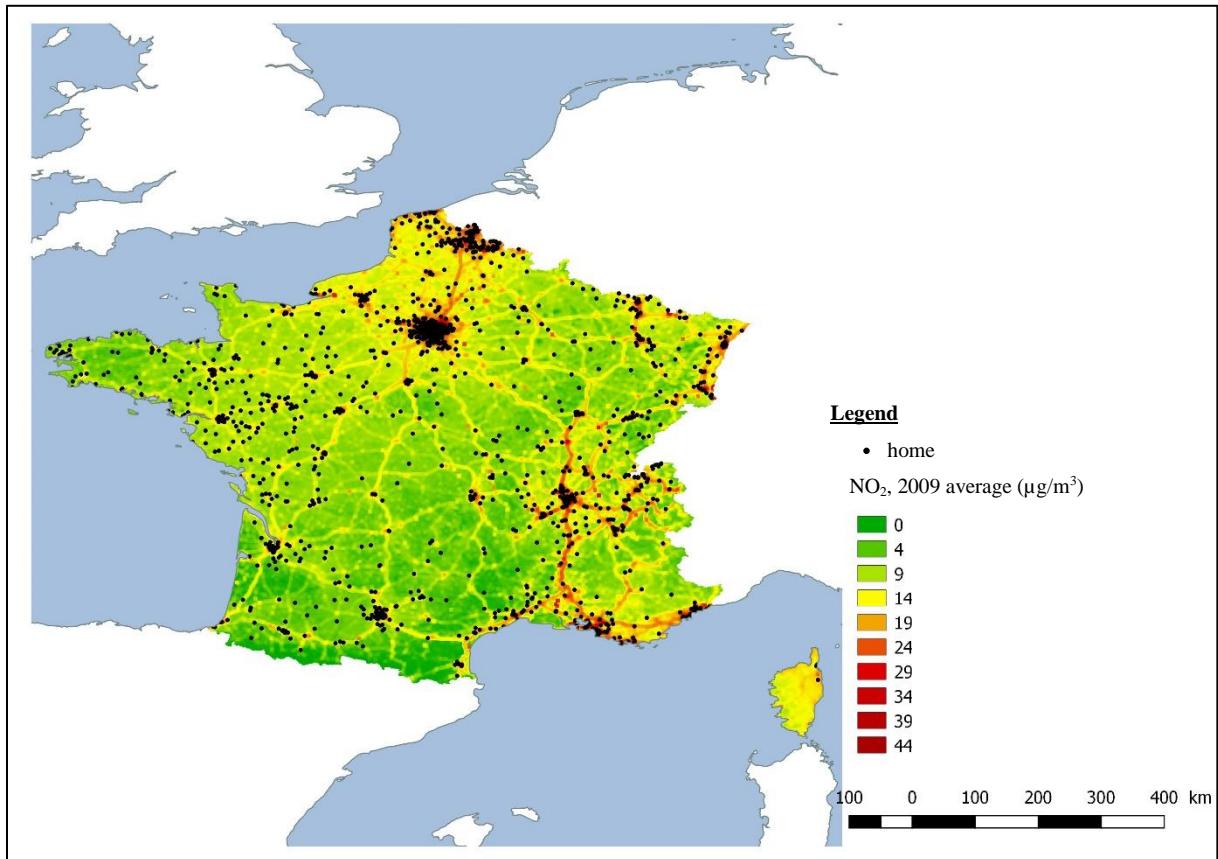


Figure IV-2: Flow chart



**Figure IV-3: Locations of home addresses of the 544 women included in the current duration analysis with levels of NO<sub>2</sub> estimated in year 2009 with the air pollution model.**



### III. Supplemental material

#### Details of exposure estimation

A fine spatial exposure model for NO<sub>2</sub> and PM<sub>10</sub> with 1x1 km spatial resolution and daily temporal resolution was developed by INERIS (French national institute for industrial environment and risks), covering the whole country for years 2009-2010. The exposure model was developed by kriging daily data from the CHIMERE chemistry-transport model and using as drift daily data from air quality monitors and –for NO<sub>2</sub>– NO<sub>x</sub> emission inventory (Benmerad M, *in press*).

As the exposure windows considered occurred before the start of the period of unprotected intercourse for current duration design and before the questionnaires (Q0 or Q1) for the prevalent cohort, we were interested in exposure windows spanning from 2004 to 2008. We retrieved data from all the air quality monitoring stations of the country from ADEME (French Environment and Energy Management Agency) between January 2002 and July 2009. Each home address was linked to the nearest point of grid and to the nearest monitoring station at less than 100 km from home. To backextrapolate the exposures from the exposure model to the study period, we assumed that the spatial contrast at the country scale were similar in 2009 than during the other years of the study period (2004-2008). We estimated the ratio of the exposure model's estimate at the home address during the 70 days of 2009 corresponding to the days and months of the exposure window divided by the exposure model's estimate at the nearest station for the same 70 days period and used this spatial ratio to correct the average during the true exposure window of the daily concentrations at the nearest station. For each exposure window, exposures of women with daily data from the station used for back-extrapolation available for less than 75% of the considered exposure window were considered missing.

To detail, we assumed that if during a defined time period during year 2009 ( $dt_{2009}$ ) the exposure level at a location  $(X_1, Y_1)$  corresponds to  $x\%$  of the whole country average during  $dt_{2009}$ , then at the same time period during another year A ( $dt_A$ , with year A included in the study period), then exposure at the same location  $(X_1, Y_1)$  will also be  $x\%$  of the exposure in the whole country during  $dt_A$ , i.e.:

$$\frac{model_{dt_{2009}}(X_1, Y_1)}{model_{2009}(France)} = \frac{model_{dt_A}(X_1, Y_1)}{model_{dt_A}(France)} = x(X_1, Y_1) \quad (1)$$



Then, for any pairs of locations  $(X_1, Y_1)_i$  [the nearest point of the grid from the home address of woman  $i$ ] and  $(X_2, Y_2)_i$  [the nearest point of the grid from the nearest station for home address of woman  $i$ ] we can deduct from equation (1) that:

$$\begin{aligned} model_{dt_A}(France) &= \frac{model_{dt_A}(X_1, Y_1)_i}{model_{dt_{2009}}(X_1, Y_1)_i} model_{dt_{2009}}(France) \\ &= \frac{model_{dt_A}(X_2, Y_2)_i}{model_{dt_{2009}}(X_2, Y_2)_i} model_{dt_{2009}}(France) \\ \Leftrightarrow model_{dt_A}(X_1, Y_1)_i &= \frac{model_{dt_{2009}}(X_1, Y_1)_i}{model_{dt_{2009}}(X_2, Y_2)_i} model_{dt_B}(X_2, Y_2)_i \end{aligned} \quad (2)$$

With the exposure model with 1x1 km grid, we can estimate  $model_{dt_{2009}}(X_1, Y_1)_i$  (i.e. the exposure estimated at home address of woman  $i$  during the exposure window  $dt$  moved in 2009) and  $model_{dt_{2009}}(X_2, Y_2)_i$  (i.e. the exposure estimated at the location of nearest station from home address of woman  $i$  during the exposure window  $dt$  moved in 2009). We want to estimate  $model_{dt_i}(X_1, Y_1)_i$  (i.e. the exposure estimated at home address of woman  $i$  during the exposure window  $dt_i$  corresponding to 70 days before the start of the period of unprotected intercourse of woman  $i$  in the current duration approach), which following equation (2) corresponds to the exposure estimated at the location of the nearest station from home address of woman  $i$  during the 70 days before her period of unprotected intercourse (i.e.  $model_{dt_i}(X_2, Y_2)_i$ ) multiplied by a corrective factor defined during the 70 days of 2009 corresponding to the days and months of the exposure window ( $dt_{i,2009}$ ). We cannot know the exact value of  $model_{dt_i}(X_2, Y_2)_i$  as the exposure model was not available during  $dt_i$ , but we can approximate it using the values measured at the station located in  $(X_2, Y_2)_i$ . We averaged the daily data from the nearest station of home address of woman  $i$  during  $dt_i$  (average called  $station_{dt_i}(X_2, Y_2)_i$ ). Then, we estimate the exposure of woman  $i$  at her home address during the exposure window  $dt_i$  (called  $exposure_{dt_i}(X_1, Y_1)_i$ ) by applying the corrective factor to the exposure estimated during  $dt$  with the nearest station for home address of woman  $i$ :

$$exposure_{dt_i}(X_1, Y_1)_i = \frac{model_{dt_{i,2009}}(X_1, Y_1)_i}{model_{dt_{i,2009}}(X_2, Y_2)_i} station_{dt_i}(X_2, Y_2)_i$$





## Chapter V: After fertilization: Effects of Atmospheric pollution on preterm delivery



## I. French summary

### Introduction

La pollution atmosphérique et les conditions météorologiques sont suspectées d'être de facteurs pouvant causer des naissances prématurées (avant 37 semaines d'aménorrhée).

### Objectif

Notre objectif était de caractériser l'impact de la pollution atmosphérique et des conditions météorologiques sur le risque de naissance prématurée.

### Méthodes

Dans le cadre du projet ESCAPE, nous avons harmonisé et poolé les informations de 71 493 naissances provenant de 13 cohortes situées dans 11 pays européens. Les expositions à l'humidité, la température et la pression atmosphérique ont été estimées à l'aide d'une station de mesure par zone géographique. Pour la pollution atmosphérique, des modèles Land Use Regression ont été développés dans le cadre du projet ESCAPE pour l'ensemble des polluants considérés (oxydes d'azote -NO<sub>2</sub>, NO<sub>x</sub>; particules -PM<sub>10</sub>, PM<sub>2.5</sub>, PM<sub>2.5-10</sub>- et absorbance des PM<sub>2.5</sub>). Les estimations annuelles au(x) domicile(s) des femmes ont été back-extrapolées et saisonnalisées à l'aide d'une station de fond par zone afin d'estimer les expositions pendant la grossesse (grossesse entière censurée après 37 semaines d'aménorrhée, premier trimestre, deuxième trimestre, mois avant la naissance, semaine avant la naissance). Une régression logistique a été utilisée pour étudier les expositions pendant le premier et le deuxième trimestre de grossesse tandis qu'un modèle de survie discret a été utilisé pour les autres fenêtres d'exposition. Nous avons supposé que les conditions météorologiques pouvaient influencer sur le risque de naissance prématurée de manière non linéaire tandis que les polluants atmosphériques pourraient avoir un effet linéaire.

### Résultats

Parmi l'ensemble des 71 493 naissances, 5% étaient des naissances prématurées, avec un taux variant de 4% (Copenhague, Danemark) à 13% (Héraklion, Grèce). Le risque de naissance prématurée avait tendance à augmenter linéairement avec le niveau de pression atmosphérique au premier trimestre (Odds Ratio -OR- pour une augmentation de 5 mBar : 1.06, intervalle de confiance -IC- à 95% [1.01 ;1.11]) sans que l'on puisse distinguer la pression atmosphérique de l'altitude. Nous avons aussi observé une tendance à une augmentation du risque de naissance prématurée lorsque la température pendant le premier trimestre de grossesse se situait entre -5

et 15°C (codage en spline,  $p=0.08$ ). Les polluants atmosphériques n'étaient pas associés au risque de naissance prématurée dans cette étude.

### Discussion

Les forces de cette étude sont l'utilisation de cohortes permettant d'avoir des informations détaillées sur les grossesses (tabac, césariennes) comparé aux registres de naissances, l'estimation des niveaux de polluants atmosphérique de façon harmonisée avec un modèle permettant d'avoir une résolution spatiale et temporelle fine et l'utilisation d'un modèle de survie avec des variables dépendantes du temps. Parallèlement, les faiblesses sont la non prise en compte du budget espace-temps des sujets et le manque d'informations permettant de distinguer différents types de naissances prématurées. Nous avons montré que dans le cadre de l'étude du risque de naissance prématurée, l'utilisation d'une régression logistique pour les fenêtres d'exposition dont la durée est différente entre les enfants nés avant ou à terme peut mener à des résultats biaisés en particulier pour le troisième trimestre et la grossesse entière.

## II. Article

This is a pre-copyedited, author-produced version of an article accepted for publication in *American Journal of Epidemiology* following peer review. The version of record “The Influence of Meteorological Factors and Atmospheric Pollutants on the Risk of Preterm Birth. *Am J Epidemiol* (2017 Jan 13) 185 (4): 247-258. doi: 10.1093/aje/kww141.” is available online at: <https://doi.org/10.1093/aje/kww141>.

### **The Influence of Meteorological Factors and Atmospheric Pollutants on the Risk of Preterm Birth**

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Abbreviations

CI: Confidence Interval

ESCAPE: European Study of Cohorts for Air Pollution Effects

NO<sub>x</sub>: Nitrogen oxides

NO<sub>2</sub>: Nitrogen dioxide

OR: Odds-Ratio

PM<sub>10</sub>: Particulate Matter with an aerodynamical diameter below 10 µm

PM<sub>2.5</sub>: Particulate Matter with an aerodynamical diameter below 2.5 µm

Running head:

Meteorological factors, air pollution and preterm birth

## 1. Abstract

Atmospheric pollutants and meteorological conditions are suspected causes of preterm birth. We aimed to characterize their possible association with preterm birth (before 37 completed gestational weeks) risk. We pooled individual data from 13 birth cohorts in 11 European countries (71,493 births from 1994 to 2011, ESCAPE project). City-specific meteorological data from routine monitors were averaged over time windows spanning from a week to the whole pregnancy. Atmospheric pollution measurements (nitrogen oxides, particulate matter) were combined with data from permanent monitors and land-use data into seasonally-adjusted Land Use Regression models. Preterm birth risks associated with air pollution and meteorological factors were estimated by adjusted discrete time Cox models. The frequency of preterm birth was 5.0%. Preterm birth risk tended to increase with atmospheric pressure first trimester average (odds ratio for each increase by 5mBar, 1.06, 95% confidence interval 1.01, 1.11), which could not be distinguished from altitude. There was also some evidence of an increase in preterm birth risk with temperature first trimester average in the -5 to 15°C range, with a plateau afterwards (spline coding,  $P=0.08$ ). No evidence of adverse association with atmospheric pollutants was observed. Our study lends support for an increase in preterm birth risk with atmospheric pressure.

Key words: Atmospheric pollution; Atmospheric pressure; Cohort; Humidity; Meteorological conditions; Pooled analysis; Preterm birth; Temperature

## 2. Introduction

Preterm birth is the adverse pregnancy outcome entailing the largest health burden on the short and long terms (1). Besides maternal smoking (2), suspected modifiable risk factors include phthalates esters (3), atmospheric pollutants and meteorological conditions.

Studies reporting a detrimental association between air pollution and preterm birth (reviewed e.g. by 4, 5) relied on various designs such as birth-records-based cohort-type (6-8) and time-series analyses (9, 10), case-controls studies (11), register-based, and a natural experiment (12). Many of these studies were conducted in the USA, where preterm delivery incidence is about twice as high as in Western Europe, and may thus have a different etiology. Overall, only very few of these studies relied on cohorts, which allow efficient control for confounders. Few of the cohort-type studies used survival modeling (13), which is the efficient way to characterize associations of time-varying exposures with survival outcomes (14, 15).

Research also suggested short-term associations of temperature with preterm birth risk (16-19). Atmospheric pressure has little been considered, nor were exposures windows of a trimester or more. Meteorology is a strong determinant of daily air pollution level. Any association between atmospheric pollutants and preterm birth risk can therefore be confounded by meteorological factors. Few studies of associations between air pollutants and preterm birth were corrected for meteorological factors (7, 20).

Our aim was to characterize the association of atmospheric pollutants and meteorological factors with preterm birth in European cohorts. Our a priori hypotheses were that atmospheric pollutants could have a (monotonic) influence on preterm birth risk, and that temperature could influence preterm birth risk, in a possibly non-monotonic way.

### 3. Methods

#### ***Study population***

We focused on cohorts of pregnant women and newborns included in ESCAPE (European Study of Cohorts for Air Pollution Effects) project, described elsewhere (21, 22). Duisburg cohort was not considered here because preterm births had not been recruited. We included 13 cohorts from 11 European countries (ABCD, Amsterdam, The Netherlands; APREG, Gyor, Hungary; BAMSE, Stockholm area, Sweden; BiB, Bradford, England; DNBC, Copenhagen area, Denmark; EDEN, Nancy and Poitiers, France; GASPII, Rome, Italy; Generation R, Rotterdam, The Netherlands; INMA (5 centers: Asturias, Gipuzkoa, Granada, Sabadell and Valencia), Spain; KANC, Kaunas, Lithuania; MOBA, Oslo area, Norway; PIAMA (3 centers, Northern, middle and Southern part of the Netherlands) and RHEA, Heraklion, Greece; Figure V-1). Recruitment periods spanned between 1994 and 2010. Included women had to have delivered a live birth and to have their home address during pregnancy located in areas where air pollution models were developed as part of ESCAPE project. Data were transferred to Inserm (Grenoble) where they were harmonized and pooled (21). We included only singleton newborns. When women had several pregnancies during the study period, we included only the first one.

#### ***Health outcome***

Preterm births (a birth before 37 completed gestational weeks) were identified relying on gestational duration based on conception date estimated from the last menstrual period whenever possible (23); otherwise (38% of births), we used by order of decreasing preference the ultrasound-based estimate or gestational duration from birth records. When the discrepancy between last menstrual period-based gestational duration (or the information from birth records) and the ultrasound-based estimate was 3 weeks or more, we modified values assuming the ultrasound-based estimate was correct. Information on Caesarean sections was not available in all cohorts. In sensitivity analyses, we focused on cohorts in which information on the occurrence of a Caesarean section was available (excluding ABCD, APREG and KANC cohorts) and repeated analyses excluding pregnancies ending with a planned Caesarean section, or for which information on whether the Caesarean section was planned was missing.

***Exposure assessment***

*Meteorological parameters:* Outdoor temperature, humidity and atmospheric pressure at the altitude of the city were defined from the hourly measures of a single monitoring station in each center and averaged during several temporal windows. Data on atmospheric pressure were not available for KANC cohort. The exposure windows considered were trimester 1 of pregnancy (from day 14 – counting from the last menstrual period – to day 105), trimester 2 (from day 106 to day 197), as well as 1-week, 4-week and whole pregnancy exposure windows (see statistical modeling). Exposure levels after gestational week 37 (hence after the considered outcome) were not considered. Exposures during the third trimester, a period during which (preterm) deliveries occur, were only considered through the 1- and 4-week exposure windows analyses.

*Air pollution and traffic indicators:* Land-Use Regression models have been developed (24, 25), allowing estimation of annual mean concentrations of ambient particulate matter (PM) with an aerodynamic diameter below 2.5 (PM<sub>2.5</sub>) and 10 µm (PM<sub>10</sub>), coarse PM (PM<sub>2.5-10</sub>), PM<sub>2.5</sub> absorbance (a proxy of black carbon PM content), nitrogen dioxide (NO<sub>2</sub>), and nitrogen oxides (NO<sub>x</sub>) at the maternal home addresses. For budgetary reasons, particulate matter levels were assessed in a subgroup of cohorts (Figure V-1). Exposure corresponded to the time-weighted average of exposure at all addresses during the exposure window considered if information on changes of address was available, and to the address at inclusion or birth when information on successive addresses had not been collected. We performed sensitivity analyses restricted to women who had not changed home address during pregnancy (or for whom all addresses were known) in the subgroup of cohorts for which this information was available.

Land-use regression models were temporally-adjusted using an approach relying on city-specific routine monitoring stations, allowing to obtain estimates of exposure relevant to each exposure window (21, 26, 27).

Traffic density on the street nearest to the maternal home address and total traffic load on major roads within a 100 m distance were also estimated (21).

### *Statistical modeling*

Unless otherwise specified, analyses were conducted pooling all cohorts. The associations of first- and second trimester exposures with preterm birth risk were assessed in distinct adjusted logistic regression models with a random effect for study center (Stata 12, College Station, TX; *xtlogit* function). Studying the association between exposures whose value may change with the duration of the pregnancy and preterm birth risk requires survival modeling (14, 15). For week-, month-specific and whole pregnancy exposures, we used a discrete time Cox model (logistic link) with birth (censored at 37 gestational weeks) as the outcome and week as the discrete time variable. Time-varying exposures (meteorological conditions and air pollutants) allowed characterizing the adjusted association between the risk of birth in a given week (before 37 gestational weeks) and exposure in the previous week, month, or since conception (whole pregnancy exposure). We compared the shape of the association between temperature whole pregnancy level and preterm birth risk estimated either with our discrete time Cox model and with a logistic model, the latter being unable to accommodate time-varying exposures in the context of at-risk periods differing between cases and non-cases (i.e. term births), possibly leading to bias.

*Adjustment factors:* For air pollution estimates, we reported the estimates of unadjusted models with a random effect for center (M1), of models adjusted for all a priori selected potential confounders excluding (M2) and including (M3) meteorological factors. Meteorological factors were adjusted for using the time window when their association with the outcome was strongest (which was not necessarily the same as the one considered for atmospheric pollutants). Air pollution levels were coded using continuous variables and estimates were reported for a priori defined increments (21). Models for meteorological factors were not adjusted for air pollutants, which we considered to be possible consequences of meteorological conditions. We used restricted cubic spline coding (28) for meteorological parameters, and tested deviation from linearity through a likelihood test. When there was no evidence of deviation from linearity, we additionally used a linear coding of meteorological factors; in the case of a V-shape relation, we used a broken stick (i.e. piecewise linear) coding (29) with a single knot located at the apparent change in slope. Center-specific analyses with subsequent random effect meta-analyses were conducted as sensitivity analyses, as well as analyses focusing on very preterm birth risk (before 32 completed gestational weeks).



#### 4. Results

##### ***Study population***

Preterm birth prevalence was 5.0% (3,533 out of 71,493 births), ranging from 3.9% (Copenhagen, Denmark) to 12.7% (Heraklion, Crete; Table V-1). Adjusted odds-ratios (OR) of preterm birth associated with maternal smoking were 1.3 (95% confidence interval, CI, 1.1, 1.4), 1.3 (1.1, 1.6) and 1.6 (1.2, 2.0), for women smoking 1-5, 6-10 and more than 10 cigarettes per day during the second trimester of pregnancy, respectively, compared to non-smoking women.

##### ***Meteorological factors and preterm birth***

The distributions of meteorological variables are shown in Table V-1 and Figure S V-1 A-C, and their correlations in Table S V-1. Between-city variations explained 15%, 48% and 95% of the variability in first trimester temperature, humidity and pressure, respectively.

Adjusted restricted cubic spline models were not strongly in favor of an association between temperature and preterm birth risk (Figure S V-2). The exposure window with the strongest association was the first trimester of pregnancy ( $P=0.08$ ). Preterm birth risk tended to increase when first trimester temperature increased from  $-5^{\circ}\text{C}$  to approximately  $10^{\circ}$  (Figure S V-2-B). A broken stick coding with a knot at  $10^{\circ}$  yielded adjusted ORs of preterm birth of 1.03 for each increase by  $1^{\circ}\text{C}$  in first trimester temperatures below  $10^{\circ}\text{C}$  (95% CI, 1.01, 1.04) and of 0.99 for each increase by  $1^{\circ}$  above  $10^{\circ}$  (95% CI, 0.97, 1.01). Meta-analytical results were similar (Figure S V-3). When first trimester temperature was coded in categories, the ORs of preterm birth were 1.13 (95% CI, 1.00, 1.27), 1.14 (0.99, 1.33) and 1.20 (0.99, 1.45) for temperatures in the  $5\text{-}10^{\circ}$ ,  $10\text{-}15^{\circ}$  and  $\geq 15^{\circ}\text{C}$  ranges, respectively, temperatures below  $5^{\circ}$  being the reference ( $P$  for trend, 0.08).

Associations between whole pregnancy temperature and preterm birth risks estimated with a survival model as above were weak ( $P=0.45$ ), with an inverse U-shape, and strongly differed from estimates of a logistic model, which were U-shaped and stronger ( $P<5.10^{-3}$ ), a manifestation of a bias in the logistic modeling approach (Figure S V-4).

There was no evidence of an association between humidity and preterm birth risk, whatever the time window considered ( $P>0.20$ , Figure S V-5).

The time window corresponding to the strongest association of atmospheric pressure with preterm delivery was the first trimester of pregnancy (Figure S V-6). The association corresponded to a monotonous increase (Figure V-2-C, test of deviation from linearity,  $P=0.20$ ).

The OR of preterm delivery was 1.06 for each increase by 5 mBar in first trimester atmospheric pressure (95% CI, 1.01, 1.11). This association was not altered after adjustment for temperature and humidity (OR, 1.07), for fine particulate matter first trimester level (OR, 1.06), after exclusion of INMA Granada center (the center with the highest altitude; OR, 1.07), after restriction to pregnancies about which we knew it was a normal delivery or an unplanned Caesarean section (n=45,135; OR, 1.06), nor after restriction to women for which information on gestational duration based on early ultrasound measurements and on last menstrual period were simultaneously available (n=27,058) and reliance on the ultrasound-based (OR, 1.06) or the last menstrual period-based definitions (OR, 1.07). It was similar after restrictions to cohorts with information on changes of address during pregnancy and exclusion of women who changed address (OR, 1.05, 95% CI, 1.00, 1.10).

There were 429 very preterm births (0.6%). The OR of very preterm delivery associated with atmospheric pressure first trimester average was similar to that corresponding to preterm birth risk, with a wider CI (1.06 for each increase by 5 mBar, 95% CI, 0.97, 1.16). Adjusted models were also in favor of an increased risk of very preterm birth with humidity in the previous week (continuous coding of humidity, P=0.05) and atmospheric pressure in the previous week (restricted cubic spline coding, P=0.04, Figure S V-7) but not with temperature (restricted cubic spline coding, all P>0.3).

### ***Air pollution and preterm birth***

The distributions of the atmospheric pollution levels are shown in Figure S V-1-D-F, and their correlations with meteorological variables in Table S V-2 and Table S V-3. There was no evidence of increased risk of preterm birth in association with any of the pollutants of interest averaged during all time windows considered, nor with traffic variables (Table V-2). Estimates from fully adjusted models corresponded to a decreased preterm delivery risk in association with nitrogen oxides (Table V-2). Analyses restricted to cohorts with information allowing to exclude planned Caesarean sections yielded similar conclusions, with point estimates associated with nitrogen oxides closer to the null association (Table S V-4). Conclusions from meta-analyses for first trimester exposure window were qualitatively similar to those of the pooled analyses, and were in favor of between-center heterogeneity in estimates (Figure S V-8).

## 5. Discussion

Our pooled analysis of 13 European cohorts supports an association between atmospheric pressure and preterm birth risk. There was some evidence for temperature in the  $-5^{\circ}\text{C}$  to  $10^{\circ}$  range being positively associated with preterm birth risk and little evidence of associations of humidity and atmospheric pollutants at the levels observed in these urban areas.

The main strengths of our study include the cohort design, the harmonized and fine-scale spatial and temporal air pollution modeling, the ability to control for a large range of potential confounders, the consideration of bias resulting from planned Caesarean-sections, and the use of a survival model. Weaknesses include the fact that atmospheric pollutants and meteorological factors exposure metrics did not incorporate the subjects' time-space activity nor the indoor levels, and our inability to distinguish preterm births in terms of associated maternal conditions (e.g., preeclampsia, infection).

Preterm birth rate was 5% in our study, which is typical for Western European areas, and is much lower than in the USA, where the rate was 12% in 2010 (30). Consequently, preterm birth in the USA and in Western Europe could be seen as two distinct pathological entities, with possibly distinct risk factors, limiting comparison between our study and US studies.

Many of the studies considering possible effects of meteorological conditions on preterm birth (16, 19)(17, 18) focused on exposures shortly before birth. These are most efficiently studied in the context of survival (or case-crossover) analyses. In a survival analysis of about 101,000 births in Australia, increases in four-week temperature averages in the  $15$  to  $25^{\circ}\text{C}$  range were associated with an increase in preterm birth risk. In a case-crossover analysis in California, in which the 5<sup>th</sup> percentile of apparent temperature averaged over 6 days was  $14.5^{\circ}\text{C}$ , short-term variations in apparent temperature were associated with an increased risk of preterm birth risk (19). Our study focused on a lower temperature range and, if anything, highlighted possible associations with temperature during the first trimester time window (which was not considered in the California study, due to its case-crossover design, nor in the Australia study), for temperature lower than in the Australia and California studies.

Living at a high altitude (entailing a lower atmospheric pressure) during pregnancy is a cause of low birth weight (31). A study in Peru reported an odds-ratio of preterm birth of 1.2 (95% CI, 1.0 to 1.5) for women living at an altitude of 3000 m or more compared to less than 2000 m (32). The study in Peru did not consider the 0-690 m altitude range in focus in our study. In a report of airplane transfers of women at risk for imminent preterm delivery but not yet in labor during transfer, Akl et al. observed that the airplane reaching an altitude above 4270 m or cabin pressure corresponding to an altitude above that of sea level (hence a decreased atmospheric pressure) was associated with a delayed time from landing to delivery, which, this time, is in favor of a short term association between low atmospheric pressure and decreased preterm birth risk (33). Given this limited literature, the issue of atmospheric pressure and altitude associations with preterm birth risk warrants further investigation.

The proximal causes of preterm delivery, a highly heterogeneous condition, include inflammatory processes at the maternal-fetal interface, infections, ischemic placental dysfunction, maternal hypertension and preeclampsia, placental abruption, preterm premature rupture of the membranes. Many of these conditions may actually be influenced by meteorology-related factors. For example, temperature, which has a clear influence on cardiac function and blood pressure outside the context of a pregnancy (34, 35), may also influence the maternal cardiovascular function of pregnant women (36, 37). Such changes in cardiac and endothelial function may in turn contribute to placental abruption, preeclampsia or ischemic placental dysfunction. In support of this hypothesis, first trimester temperature levels have been associated with the risk of severe preeclampsia (36). The frequency of vaginal infections may vary with temperature and season (38) and some of these infections may, directly or in association with preterm premature rupture of the membranes, lead to a preterm delivery (39).

A meta-analysis of air pollution associations with preterm birth risk showed heterogeneity in the exposure windows reported in each study (5, Figure 4), suggesting selective reporting of associations within studies; publication bias was also highlighted (5). This meta-analysis reported odds-ratios of preterm delivery of 0.97 and 0.95 for an increase by 20  $\mu\text{g}/\text{m}^3$  in  $\text{PM}_{10}$  first and second trimester concentrations, respectively, which would correspond to 0.98 and 0.97 for a 10  $\mu\text{g}/\text{m}^3$  increase in  $\text{PM}_{10}$ , very close to our adjusted hazard rate of 0.98 for both windows. The meta-analytical estimate corresponded to an increased risk of preterm birth for

the whole pregnancy (OR, 1.35) and third trimester (1.06) exposure windows (5). These exposure windows are those for which the bias related to the preterm and term births having exposure windows of different lengths may happen in studies based on logistic modeling – indeed our analysis (see Figure S V-4) indicated much stronger associations with temperature whole pregnancy average with a logistic model than with our survival modeling approach. Such a bias is also expected for third trimester exposures and for other seasonally-varying factors such as atmospheric pollutants. For the whole pregnancy window, our study was not in favor of an increased risk associated with any pollutant. For nitrogen oxides, estimates unexpectedly tended to correspond to a protective association for some exposure windows, a trend that weakened in analyses restricted to spontaneous preterm births and unplanned Caesarean sections (Table S V-4). A recent large New-York study reported similar trends for protective associations (40). Moreover, our meta-analysis was in favor of between-city heterogeneity for associations with particulate matter. This could be explained by between-city heterogeneity in particulate matter composition, and in heterogeneity of associations between each specific particulate matter chemical component and preterm birth, an issue so far little considered (41).

Studies suggested associations of atmospheric pollutants with preeclampsia risk (8) and blood pressure in pregnant women (42-44). This might imply that any effect of air pollutants on preterm birth risk is restricted to preterm births with a hypertensive etiology. However, the data available in this and in most former studies did not allow such detailed analyses of air pollution influences on specific subtypes of preterm deliveries.

To our knowledge, few previous studies of preterm birth considered the possible confounding role of meteorological factors in the estimated effect of atmospheric pollutants (16, 19). In a case-crossover analysis of 16 California counties, Basu et al (19) did not identify a significant short-term association of ozone, nitrogen dioxide, sulfur dioxide or fine particulate matter concentrations on preterm delivery risk independently of meteorological factors. In a birth register-based study of 101,870 births in Australia, Strand et al. (16) described the association of meteorological factors with preterm birth risk. Associations with atmospheric pollutants were not reported, and the adjustment for sulfur dioxide levels did not modify the associations between meteorological factors and occurrence of a live birth (16).

In terms of exposure assessment of atmospheric pollutants, our model included both a (land-use regression-based) spatial component and a temporal component based on monitoring stations (21, 26). Information on change of address was known for 11 cohorts, in which 15% of women moved during pregnancy, and sensitivity analyses were not in favor of lack of consideration of changes in home address inducing a strong bias. More importantly, only outdoor levels at the home address were considered. This issue also applies to temperature (and, to some extent, humidity), for which the outdoor levels assessed in meteorological networks constitute a poor proxy of the average temperature to which the woman is exposed, given that people spend most of their time indoors and have different heating and window opening habits.

## 6. Conclusions

Our study highlighted an increased risk of preterm birth in association with atmospheric pressure (which could not be distinguished from altitude). Regarding temperature, if anything, preterm birth risk tended to increase with first trimester temperatures in the range between  $-5^{\circ}$  and  $10^{\circ}\text{C}$ . Results were not in favor of short-term (week to month) associations with temperature averages in late pregnancy, although our power to discard such associations was limited. This study did not bring additional evidence regarding an association between atmospheric pollutants at levels currently encountered in European urban areas and preterm birth risk. Future studies investigating associations between atmospheric pollutants and preterm birth should carefully correct for meteorological factors, which constitute potential confounders, rely on models allowing avoiding considering exposure windows of different lengths for preterm and term births, and consider collecting information on maternal, fetal and placental conditions to distinguish preterm birth cases with different proximal etiology.

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## CONFLICT OF INTEREST

None.

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## 9. Tables and figures

**Table V-1: Characteristics of the Study Population (N=71,493 Live Births from 13 European Cohorts part of ESCAPE project, 1994-2010).**

Characteristics	Mean (5 <sup>th</sup> -95 <sup>th</sup> Percentiles)	Total population	Preterm birth (5%)		Term birth (95%)		<i>P</i> <sup>a</sup>
			%	Mean (5 <sup>th</sup> -95 <sup>th</sup> Percentiles)	%	Mean (5 <sup>th</sup> -95 <sup>th</sup> Percentiles)	
Maternal age (years)							<10 <sup>-3</sup>
<25		10,512	5.4		94.6		
25-29		23,217	4.8		95.2		
30-34		26,069	4.5		95.5		
35-39		10,122	5.5		94.5		
≥ 40		1,496	7.9		92.1		
Maternal education							0.001
Low		13,667	5.5		94.5		
Intermediate		25,929	5.0		95.0		
High		28,742	4.6		95.4		
Mother living alone							<10 <sup>-3</sup>
No		62,682	4.9		95.1		
Yes		3,250	7.5		92.5		
Parity							<10 <sup>-3</sup>
0		37,701	5.6		94.4		
1 previous child		22,744	4.0		96.0		
≥ 2 previous children		10,448	4.8		95.2		
Sex of offspring							<10 <sup>-3</sup>
Male		36,524	5.3		94.7		
Female		34,969	4.5		95.5		
Maternal smoking (2 <sup>nd</sup> trimester)							<10 <sup>-3</sup>
No		59,613	4.7		95.3		
1-5 cig. /day		5,897	5.9		94.1		
6-10		2,523	5.8		94.2		
≥ 10		1,240	7.2		92.8		
Maternal height (cm)							<10 <sup>-3</sup>
<160		9,747	6.2		93.8		
160-169		34,427	5.1		94.9		
≥ 170		25,956	4.2		95.8		
Maternal weight (kg) <sup>a</sup>							<10 <sup>-3</sup>
<50		2,357	7.3		92.7		
50-59		18,593	5.2		94.8		
60-69		25,000	4.5		95.5		
70-79		12,692	4.4		95.6		
≥ 80		9,303	5.4		94.6		
Pregnancy-related hypertension							<10 <sup>-3</sup>
No		50,971	4.6		95.4		
Yes		4,549	8.2		91.8		

Effects of Atmospheric pollution on preterm delivery

Characteristics	Mean (5 <sup>th</sup> -95 <sup>th</sup> Percentiles)	Total population	Preterm birth (5%)		Term birth (95%)		P <sup>a</sup>
			%	Mean (5 <sup>th</sup> -95 <sup>th</sup> Percentiles)	%	Mean (5 <sup>th</sup> -95 <sup>th</sup> Percentiles)	
Caesarean section							<10 <sup>-3</sup>
No		48,977	3.8		96.2		
Yes		8,533	11.0		89.0		
Season of conception							0.016
January-March		16,680	4.9		95.1		
April-June		15,928	5.4		94.6		
July-September		18,314	4.8		95.2		
October-December		20,571	4.7		95.3		
Country							<10 <sup>-3</sup>
Norway		10,307	4.8		95.2		
Sweden		3,870	4.4		95.6		
Denmark		17,169	3.9		96.1		
Lithuania		4,087	5.6		94.4		
England		9,898	5.6		94.5		
The Netherlands		19,105	5.0		95.0		
France		1,286	5.8		94.2		
Hungary		1,290	6.8		93.2		
Italy		684	5.0		95.1		
Spain		2,620	4.2		95.8		
Greece		1,177	12.7		87.3		
Temperature (°C) <sup>b</sup>							<10 <sup>-3</sup>
<5		9,812	4.3		95.7		
5-9.9		31,558	4.8		95.2		
10-14.9		25,922	5.1		94.9		
≥ 15		3,443	7.6		92.4		
Mean <sup>c</sup> (5-95 <sup>th</sup> centiles), °C	9.1 (3.2-14.9)			9.6 (3.4-17.4)		9.1 (3.2-14.9)	
Humidity (%) <sup>b</sup>							<10 <sup>-3</sup>
<70		8,346	6.2		93.8		
70-74.9		11,216	4.6		95.4		
75-79.9		20,000	4.4		95.6		
80-84.9		19,916	4.9		95.1		
≥ 85		12,015	5.4		94.6		
Mean <sup>c</sup> (5-95 <sup>th</sup> centiles), %	78 (65-89)			78 (62-89)		78 (65-89)	
Atmospheric pressure, mBar <sup>b</sup>							<10 <sup>-3</sup>
<1010		44,284	4.6		95.4		
1010-1012.9		2,126	8.6		91.4		
1013-1015.9		9,046	5.4		94.6		
≥ 1016		11,289	5.0		95.0		
Mean <sup>c</sup> (5-95 <sup>th</sup> centiles), mBar	1004 (981-1018)			1004 (981-1018)		1004 (981-1018)	

<sup>a</sup> P-values are from  $\chi^2$  tests.

<sup>b</sup> Before pregnancy.

<sup>c</sup> Average between fertilization date and the end of the 32<sup>nd</sup> gestational week.

**Table V-2: Associations Between Atmospheric Pollutants and Preterm Birth (Pooled Analysis of 13 European Cohorts part of ESCAPE project, 1994-2010).**

Pollutant and exposure window	Unadjusted OR (M1)			Partly adjusted OR (M2) <sup>a</sup>			Fully adjusted OR (M3) <sup>b</sup>		
	N	OR <sup>c</sup>	95% CI	N	OR <sup>c</sup>	95% CI	N	OR <sup>c</sup>	95% CI
<b>NO<sub>2</sub></b>									
Whole pregnancy <sup>d</sup>	69,503	0.97	0.93, 1.01	62,127	0.96	0.92, 1.01	56,977	0.96	0.91, 1.01
1 <sup>st</sup> trimester	68,042	0.96	0.93, 1.00	60,814	0.98	0.92, 1.01	55,811	0.97	0.92, 1.02
2 <sup>nd</sup> trimester	68,183	0.98	0.95, 1.02	60,947	0.96	0.92, 1.01	55,892	0.96	0.92, 1.01
Previous week <sup>e</sup>	70,210	1.01	0.98, 1.04	62,687	0.99	0.95, 1.02	57,534	0.98	0.94, 1.01
Previous month <sup>e</sup>	70,205	1.00	0.96, 1.03	62,684	0.97	0.93, 1.01	57,531	0.96	0.92, 1.00
<b>NO<sub>x</sub></b>									
Whole pregnancy <sup>d</sup>	68,215	0.98	0.93, 1.00	60,890	0.96	0.92, 1.00	55,777	0.96	0.92, 1.00
1 <sup>st</sup> trimester	66,762	0.96	0.93, 0.99	59,583	0.97	0.93, 1.00	54,619	0.97	0.93, 1.01
2 <sup>nd</sup> trimester	66,913	0.98	0.95, 1.01	59,725	0.96	0.93, 1.00	54,707	0.97	0.93, 1.00
Previous week <sup>e</sup>	68,932	1.00	0.98, 1.03	61,457	0.98	0.96, 1.01	56,341	0.98	0.95, 1.01
Previous month <sup>e</sup>	68,925	0.99	0.97, 1.02	61,452	0.97	0.93, 1.00	56,336	0.96	0.93, 1.00
<b>PM<sub>2.5</sub></b>									
Whole pregnancy <sup>d</sup>	56,139	0.96	0.89, 1.03	50,878	0.97	0.89, 1.05	46,791	0.96	0.87, 1.04
1 <sup>st</sup> trimester	55,522	0.96	0.91, 1.02	50,329	0.98	0.92, 1.05	46,242	0.98	0.91, 1.05
2 <sup>nd</sup> trimester	56,658	0.98	0.93, 1.04	51,316	0.98	0.92, 1.05	47,153	0.96	0.90, 1.03
Previous week <sup>e</sup>	57,966	1.01	0.98, 1.04	52,422	1.00	0.97, 1.03	47,776	1.00	0.96, 1.03
Previous month <sup>e</sup>	57,884	0.99	0.95, 1.04	52,350	0.98	0.93, 1.03	47,771	0.97	0.91, 1.02
<b>PM<sub>10</sub></b>									
Whole pregnancy <sup>d</sup>	56,139	0.95	0.87, 1.05	50,878	0.97	0.87, 1.07	46,791	0.97	0.87, 1.07
1 <sup>st</sup> trimester	55,522	0.97	0.90, 1.04	50,329	0.98	0.90, 1.07	46,242	0.98	0.90, 1.07
2 <sup>nd</sup> trimester	56,658	0.97	0.91, 1.05	51,316	0.98	0.90, 1.06	47,153	0.98	0.90, 1.06
Previous week <sup>e</sup>	57,966	1.00	0.96, 1.04	52,422	0.99	0.95, 1.03	47,776	0.99	0.95, 1.04
Previous month <sup>e</sup>	57,884	0.98	0.92, 1.03	52,350	0.97	0.91, 1.03	47,771	0.97	0.91, 1.03
<b>PM(coarse)</b>									
Whole pregnancy <sup>d</sup>	56,139	0.98	0.91, 1.06	50,878	0.99	0.91, 1.07	46,791	1.00	0.92, 1.08
1 <sup>st</sup> trimester	53,821	0.99	0.92, 1.06	48,874	0.99	0.91, 1.06	44,798	0.99	0.91, 1.07
2 <sup>nd</sup> trimester	54,985	0.99	0.92, 1.06	49,870	0.99	0.91, 1.06	45,725	1.00	0.92, 1.08
Previous week <sup>e</sup>	57,877	0.99	0.95, 1.03	52,346	0.99	0.95, 1.04	47,707	0.99	0.94, 1.04
Previous month <sup>e</sup>	57,499	0.97	0.92, 1.03	52,019	0.98	0.92, 1.05	47,747	0.98	0.92, 1.05
<b>PM<sub>2.5</sub> absorbance</b>									
Whole pregnancy <sup>d</sup>	57,086	0.92	0.84, 1.00	51,682	0.90	0.81, 1.00	46,846	0.92	0.82, 1.02
1 <sup>st</sup> trimester	55,764	0.91	0.85, 0.97	50,506	0.92	0.85, 1.00	45,713	0.95	0.87, 1.05
2 <sup>nd</sup> trimester	56,248	0.99	0.93, 1.06	50,967	0.97	0.89, 1.06	46,130	0.97	0.88, 1.07
Previous week <sup>e</sup>	58,194	1.03	0.98, 1.07	52,620	1.01	0.96, 1.07	47,781	0.99	0.94, 1.05
Previous month <sup>e</sup>	58,187	1.02	0.96, 1.07	52,614	0.99	0.93, 1.06	47,775	0.96	0.89, 1.04
<b>Traffic markers</b>									
Traffic density on nearest street	66,963	0.99	0.96, 1.02	59,676	0.99	0.96, 1.02	54,796	0.98	0.95, 1.02
Traffic load on major road within 100 m	68,391	0.97	0.94, 1.01	61,070	0.97	0.94, 1.01	55,913	0.96	0.89, 1.03

NO<sub>2</sub>: Nitrogen dioxide. NO<sub>x</sub>: Nitrogen oxides. OR: Odds-Ratio. PM: Particulate matter.

<sup>a</sup> Adjusted for infant sex, maternal educational level, parity (0, 1, ≥2), season of conception, maternal smoking during trimester 2 of pregnancy, maternal weight (broken stick model with a knot at 60 kg), maternal height (continuous coding), age; center was controlled for using a random effect variable (also in models M1).

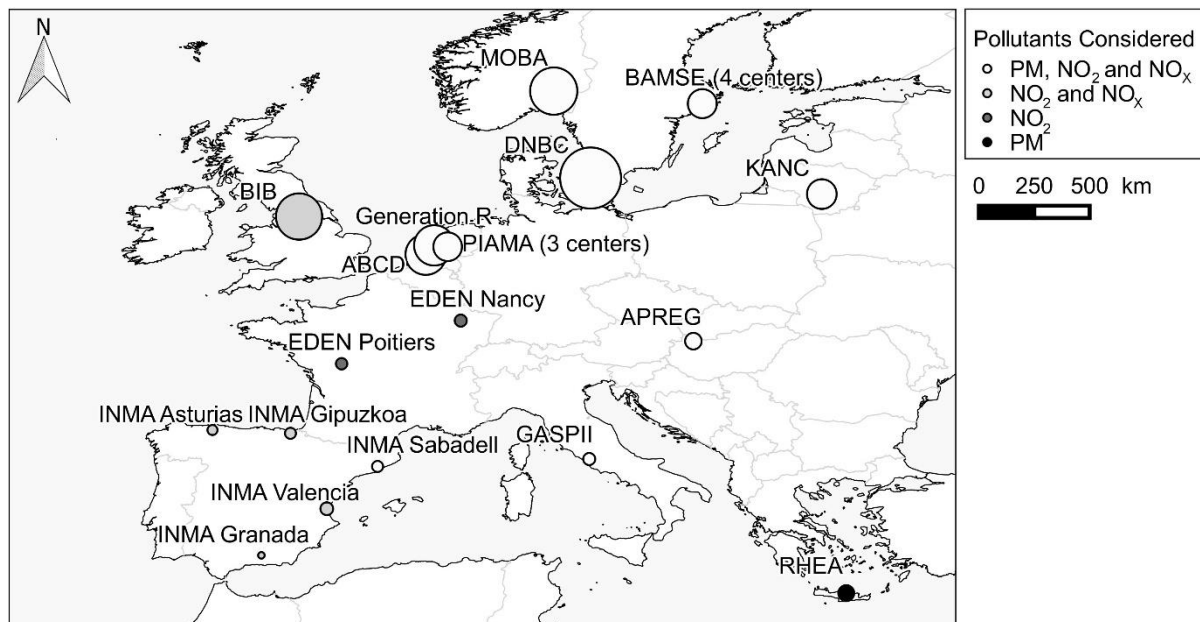
<sup>b</sup> Models M3 include all factors of M2 (see above) as well as temperature (restricted cubic spline coding) and atmospheric pressure (continuous coding) first trimester levels.

<sup>c</sup> Effect estimates are reported for an increase by 10 µg/m<sup>3</sup> in NO<sub>2</sub> and PM<sub>10</sub> concentrations, by 20 µg/m<sup>3</sup> in NO<sub>x</sub> concentrations, by 5 µg/m<sup>3</sup> in PM<sub>2.5</sub> and PM<sub>coarse</sub>, by 10<sup>-5</sup>/m in PM<sub>2.5</sub> absorbance, 5,000 vehicles per day for traffic density, and 4,000,000 vehicles per day x m for traffic load.

<sup>d</sup> Time-varying covariate averaged from gestational week 3 to birth or end of gestational week 37 (whichever came first).

<sup>e</sup> Estimated effect of weekly (respectively monthly) air pollution levels on the risk of preterm birth the following week (respectively month), as estimated from a discrete time survival model censored at 37 gestational weeks.

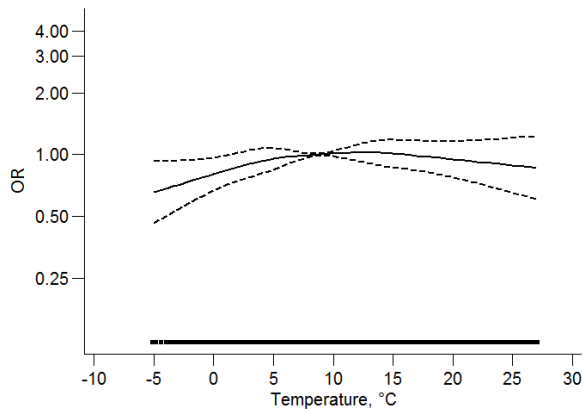
**Figure V-1: Localization of the study areas within Europe and pollutants assessed at each location. The surface of the circle is proportional to the number of subjects in each center. 13 European Cohorts part of ESCAPE project, 1994-2010.**



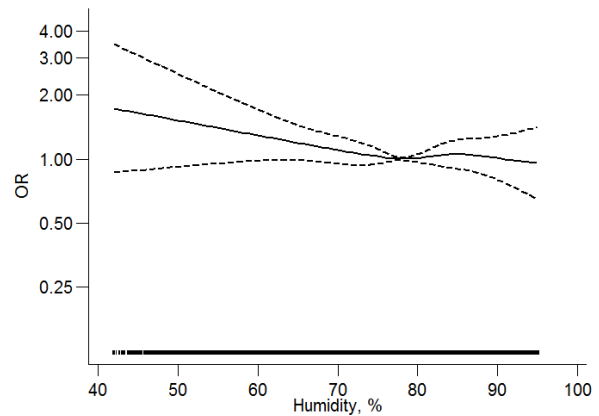
**Figure V-2: Preterm birth and A) Temperature (first trimester), B) Humidity (whole pregnancy; discrete time survival model) and C) Atmospheric pressure first trimester average.**

Restricted cubic spline models adjusted for the variables of model M2 (see Table V-2). The *P*-Value corresponds to the overall test of the spline variables in the adjusted model (13 European Cohorts part of ESCAPE project, 1994-2010). For each meteorological condition, only the exposure window corresponding to the strongest statistical association is reported. A report of associations at all exposure windows is given in Figures S-V-2, S-V-5 and S-V-6.

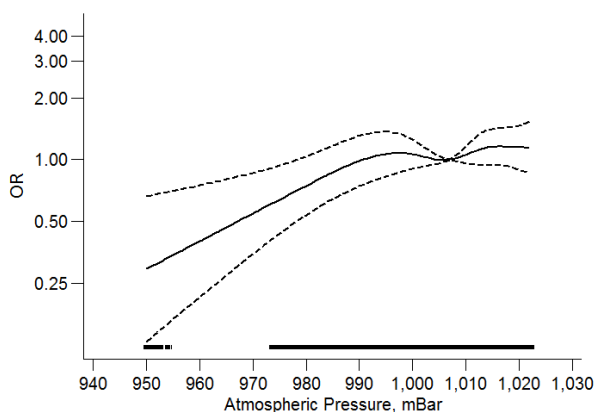
**A) Temperature (1<sup>st</sup> trimester) and preterm birth (63,158 births, *P* = 0.08)**



**B) Humidity (whole pregnancy) and preterm birth (63,910 births, *P* = 0.41)**



**C) Atmospheric pressure (1<sup>st</sup> trimester) and preterm birth (59507 births, *P* = 0.03)**





## III. Supplement material

**Summary**

- Table S V-1: Pairwise coefficients of correlation between meteorological conditions averaged during various temporal windows (13 European birth cohorts, 1994-2010). **136**
- Table S V-2: Coefficient of correlation between air pollutants and meteorological conditions (mean pregnancy levels, until gestational week 37 or birth, whichever comes first)(13 European birth cohorts, 1994-2010). **137**
- Table S V-3: Pairwise coefficients of correlation between air pollutants and meteorological conditions (averaged during the first trimester of pregnancy)(13 European birth cohorts, 1994-2010). **137**
- Table S V-4: Estimated effect of atmospheric pollutants on preterm birth, excluding planned Caesarean sections and Caesarean sections with unknown timing (pooled analysis of 10 European cohorts with information on occurrence of Caesarean sections, 1994-2010). **138**
- Figure S V-1: Distribution (boxplots) of meteorological conditions and air pollution levels (pregnancy averages censored at 37 weeks of gestation) for each center and overall (first line). **139**
- Figure S V-2: Adjusted odds-ratios (OR) of preterm delivery associated with temperature at each time window (13 European birth cohorts, 1994-2010). Restricted cubic spline models adjusted for the covariates of model M2 (see Table V-2). **141**
- Figure S V-3: Meta-analysis of the association between temperature first trimester level and preterm birth risk (13 European birth cohorts, 1994-2010). **142**
- Figure S V-4: Two estimates of the adjusted association between temperature pregnancy levels and the probability of preterm delivery (13 European birth cohorts, 1994-2010). **143**
- Figure S V-5: Adjusted odds-ratios of preterm birth associated with humidity at each time window (13 European birth cohorts, 1994-2010). **144**
- Figure S V-6: Adjusted odds-ratios (OR) of preterm birth associated with atmospheric pressure at each time window (13 European birth cohorts, 1994-2010). **145**
- Figure S V-7: Adjusted odds-ratios (OR) of very preterm birth (before 32 completed gestational weeks) associated **A**) with humidity the previous week **B**) atmospheric pressure the previous week. **146**
- Figure S V-8: Meta-analyses of the association between atmospheric pollutants and preterm birth risk (13 European birth cohorts, 1994-2010). **147**

**Table S V-1: Pairwise coefficients of correlation between meteorological conditions averaged during various temporal windows (13 European birth cohorts, 1994-2010).**

	Temperature			Humidity			Pressure		
	Preg.	T1	T2	Preg.	T1	T2	Preg.	T1	T2
Temperature									
Whole pregnancy	1								
1 <sup>st</sup> trimester	.45	1							
2 <sup>nd</sup> trimester	.91	.15	1						
Humidity									
Whole pregnancy	-.38	.07	-.40	1					
1 <sup>st</sup> trimester	-.54	-.50	-.48	.72	1				
2 <sup>nd</sup> trimester	-.37	.33	-.49	.91	.47	1			
Pressure									
Whole pregnancy	.14	.03	.09	-.23	-.16	-.19	1		
1 <sup>st</sup> trimester	.16	.07	.11	-.23	-.20	-.17	.98	1	
2 <sup>nd</sup> trimester	.13	.01	.09	-.23	-.14	-.22	.98	.95	1

All p-values are below  $10^{-3}$ . Preg.: whole pregnancy average (truncated at gestational week 37). T1 (respectively, T2): First (respectively, second) trimester of pregnancy.

**Table S V-2: Coefficient of correlation between air pollutants and meteorological conditions (mean pregnancy levels, until gestational week 37 or birth, whichever comes first)(13 European birth cohorts, 1994-2010).**

	NO <sub>2</sub>	NO <sub>x</sub>	PM <sub>2.5</sub>	PM <sub>10</sub>	PM <sub>2.5-10</sub>	PM <sub>2.5 abs</sub>	T°	Humidity
NO <sub>2</sub>	1							
NO <sub>x</sub>	0.89	1						
PM <sub>2.5</sub>	0.52	0.38	1					
PM <sub>10</sub>	0.64	0.50	0.91	1				
PM <sub>2.5-10</sub> (coarse)	0.71	0.60	0.63	0.89	1			
PM <sub>2.5</sub> absorbance	0.62	0.62	0.66	0.67	0.56	1		
Temperature	0.10	0.00 <sup>(a)</sup>	0.23	0.36	0.42	0.00 <sup>(a)</sup>	1	
Humidity	0.10	0.03	0.46	0.33	0.10	0.43	-0.38	1
Pressure	0.37	0.26	0.70	0.72	0.58	0.33	0.14	-0.23

Unless otherwise specified, all p-values are below  $5 \cdot 10^{-5}$ .

<sup>a</sup>  $p \geq 0.26$

**Table S V-3: Pairwise coefficients of correlation between air pollutants and meteorological conditions (averaged during the first trimester of pregnancy)(13 European birth cohorts, 1994-2010).**

	NO <sub>2</sub>	NO <sub>x</sub>	PM <sub>2.5</sub>	PM <sub>10</sub>	PM <sub>2.5-10</sub>	PM <sub>2.5 abs</sub>	T°	Humidity
NO <sub>2</sub>	1							
NO <sub>x</sub>	0.90	1						
PM <sub>2.5</sub>	0.55	0.43	1					
PM <sub>10</sub>	0.64	0.51	0.92	1				
PM <sub>2.5-10</sub> (coarse)	0.69	0.57	0.66	0.89	1			
PM <sub>2.5</sub> absorbance	0.69	0.71	0.66	0.66	0.56	1		
Temperature	-0.23	-0.30	-0.16	-0.03	0.07	-0.39	1	
Humidity	0.22	0.23	0.35	0.25	0.09	0.52	-0.50	1
Pressure	0.35	0.24	0.66	0.68	0.57	0.28	0.07	-0.20

All p-values are below  $5 \cdot 10^{-5}$ .

**Table S V-4: Estimated effect of atmospheric pollutants on preterm birth, excluding planned Caesarean sections and Caesarean sections with unknown timing (pooled analysis of 10 European cohorts with information on occurrence of Caesarean sections, 1994-2010).**

Pollutant and exposure window	Unadjusted effect (M1)			Partly adjusted effect (M2) <sup>(a)</sup>			Fully adjusted effect (M3) <sup>(b)</sup>					
	n	OR <sup>(c)</sup>	95% CI	n	OR <sup>(c)</sup>	95% CI	n	OR <sup>(c)</sup>	95% CI			
<b>NO<sub>2</sub></b>												
Whole pregnancy <sup>(d)</sup>	51,235	0.99	0.94	1.04	44,752	0.98	0.93	1.03	43,847	0.98	0.94	1.03
1st trimester	50,022	0.97	0.93	1.01	43,664	0.99	0.95	1.03	42,882	0.98	0.93	1.03
2 <sup>nd</sup> trimester	50,147	1.00	0.96	1.05	43,784	0.98	0.93	1.04	42,951	0.98	0.94	1.03
Previous week <sup>(e)</sup>	51,970	1.03	0.99	1.06	45,340	1.00	0.96	1.03	44,433	0.99	0.95	1.03
Previous month <sup>(e)</sup>	51,943	1.02	0.98	1.06	45,315	0.99	0.95	1.03	44,408	0.98	0.94	1.02
<b>NO<sub>x</sub></b>												
Whole pregnancy <sup>(d)</sup>	50,022	0.99	0.95	1.03	43,590	0.97	0.93	1.02	42,721	0.98	0.94	1.02
1st trimester	48,827	0.97	0.94	1.01	42,515	0.98	0.94	1.02	41,771	0.98	0.94	1.03
2 <sup>nd</sup> trimester	48,960	1.00	0.97	1.04	42,643	0.98	0.9	1.02	41,846	0.99	0.95	1.03
Previous week <sup>(e)</sup>	50,777	1.01	0.99	1.04	44,193	0.99	0.96	1.02	43,322	0.99	0.95	1.02
Previous month <sup>(e)</sup>	50,740	1.01	0.97	1.04	44,160	0.98	0.94	1.01	43,289	0.97	0.94	1.01
<b>PM<sub>2.5</sub></b>												
Whole pregnancy <sup>(d)</sup>	40,511	0.93	0.84	1.03	35,662	0.94	0.83	1.05	35,662	0.91	0.82	1.01
1st trimester	40,054	0.94	0.87	1.02	35,267	0.98	0.89	1.07	35,267	0.95	0.87	1.05
2 <sup>nd</sup> trimester	40,891	0.96	0.88	1.04	35,975	0.93	0.84	1.03	35,914	0.92	0.84	1.01
Previous week <sup>(e)</sup>	42,025	1.03	0.98	1.07	36,921	1.00	0.96	1.05	36,441	1.00	0.95	1.05
Previous month <sup>(e)</sup>	41,886	1.04	0.98	1.10	36,792	0.99	0.92	1.06	36,371	0.98	0.91	1.05
<b>PM<sub>10</sub></b>												
Whole pregnancy <sup>(d)</sup>	40,511	0.94	0.83	1.06	35,662	0.94	0.82	1.07	35,662	0.93	0.83	1.05
1st trimester	40,054	0.95	0.86	1.04	35,267	0.98	0.88	1.09	35,267	0.96	0.86	1.07
2 <sup>nd</sup> trimester	40,891	0.96	0.87	1.05	35,975	0.93	0.83	1.05	35,914	0.94	0.84	1.05
Previous week <sup>(e)</sup>	42,025	1.01	0.96	1.06	36,921	0.99	0.93	1.04	36,441	0.99	0.94	1.05
Previous month <sup>(e)</sup>	41,886	1.02	0.95	1.10	36,792	0.98	0.90	1.07	36,371	0.98	0.91	1.07
<b>PM(coarse)</b>												
Whole pregnancy <sup>(d)</sup>	40,511	0.98	0.89	1.07	35,662	0.98	0.88	1.08	35,662	0.98	0.89	1.08
1st trimester	38,485	0.98	0.90	1.07	33,924	0.98	0.89	1.89	33,924	0.98	0.89	1.08
2 <sup>nd</sup> trimester	39,326	0.98	0.90	1.07	34,618	0.97	0.87	1.07	34,558	0.99	0.89	1.09
Previous week <sup>(e)</sup>	41,820	0.99	0.94	1.04	36,750	0.98	0.93	1.04	36,276	0.98	0.93	1.04
Previous month <sup>(e)</sup>	41,311	1.00	0.93	1.08	36,302	0.98	0.91	1.07	35,886	0.99	0.92	1.07
<b>PM<sub>2.5</sub> absorbance</b>												
Whole pregnancy <sup>(d)</sup>	41,214	0.94	0.84	1.05	36,252	0.89	0.77	1.01	35,601	0.89	0.78	1.02
1st trimester	40,125	0.89	0.82	0.97	35,282	0.92	0.82	1.03	34,671	0.96	0.85	1.08
2 <sup>nd</sup> trimester	40,485	1.03	0.95	1.12	35,630	0.97	0.87	1.09	34,979	0.97	0.86	1.08
Previous week <sup>(e)</sup>	42,228	1.04	0.99	1.10	37,100	1.00	0.94	1.07	36,447	0.99	0.92	1.06
Previous month <sup>(e)</sup>	42,190	1.06	0.99	1.13	37,066	0.99	0.91	1.08	36,413	0.96	0.88	1.06

(a) Adjusted for infant sex, maternal educational level, parity (0, 1,  $\geq 2$ ), trimester of conception, maternal smoking during trimester 2, maternal weight (broken stick model with a knot at 60 kg), maternal height (continuous coding), age; center was controlled for using a random effect variable (also in models M1).

(b) Models M3 include all factors of M2 (see above) as well as temperature (restricted cubic spline) and pressure during first trimester (continuous coding).

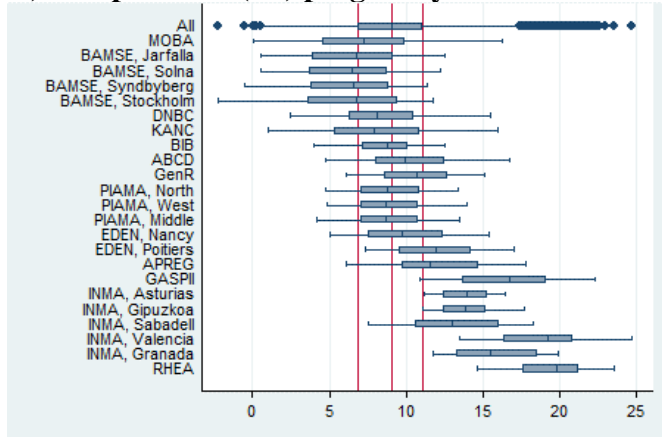
(c) Effect estimates are reported for an increase by 10  $\mu\text{g}/\text{m}^3$  in NO<sub>2</sub>, NO<sub>x</sub> and PM<sub>10</sub> concentrations, by 5  $\mu\text{g}/\text{m}^3$  in PM<sub>2.5</sub> and PM<sub>coarse</sub> and by 10<sup>-5</sup>/m in PM<sub>2.5</sub> absorbance.

<sup>d</sup> Time-varying covariate averaged from gestational week 3 to birth or end of gestational week 37 (whichever came first).

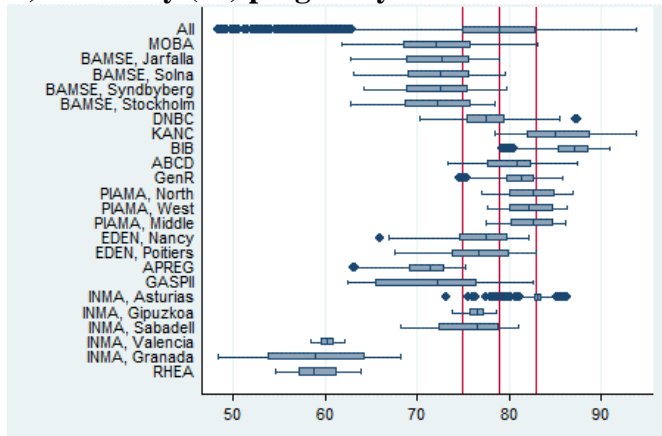
<sup>e</sup> Estimated effect of weekly (respectively monthly) air pollution levels on the risk of preterm birth the following week (respectively month), as estimated from a discrete time survival model censored at 37 gestational weeks.

**Figure S V-1: Distribution (boxplots) of meteorological conditions and air pollution levels (pregnancy averages censored at 37 weeks of gestation) for each center and overall (first line).**

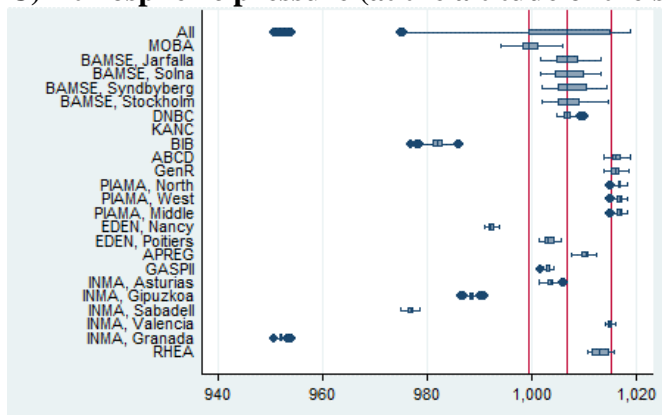
**A) Temperature (°C) pregnancy levels**



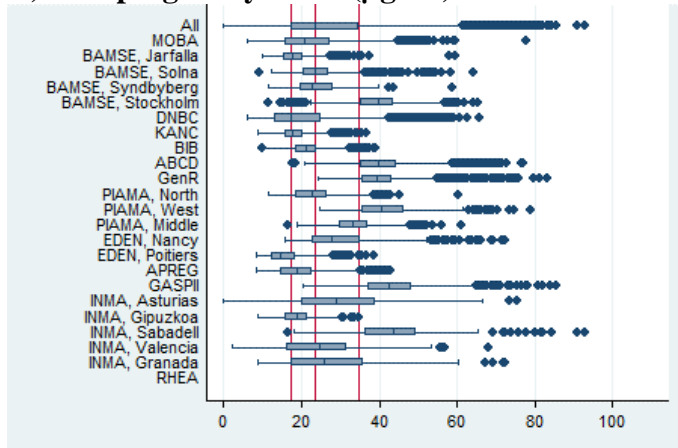
**B) Humidity (%) pregnancy levels**



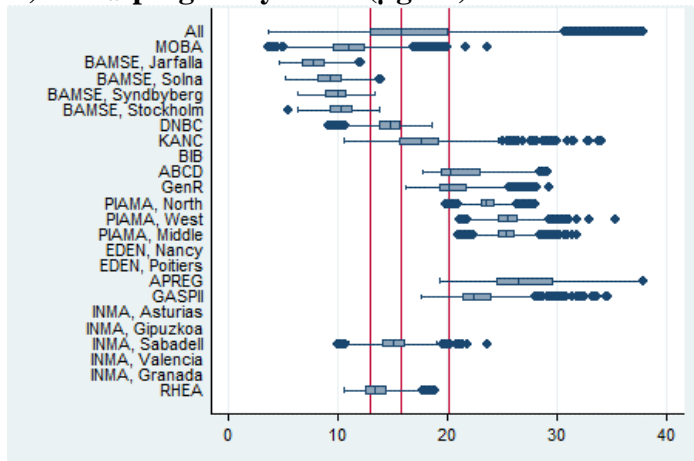
**C) Atmospheric pressure (at the altitude of the station, mBar) pregnancy levels**



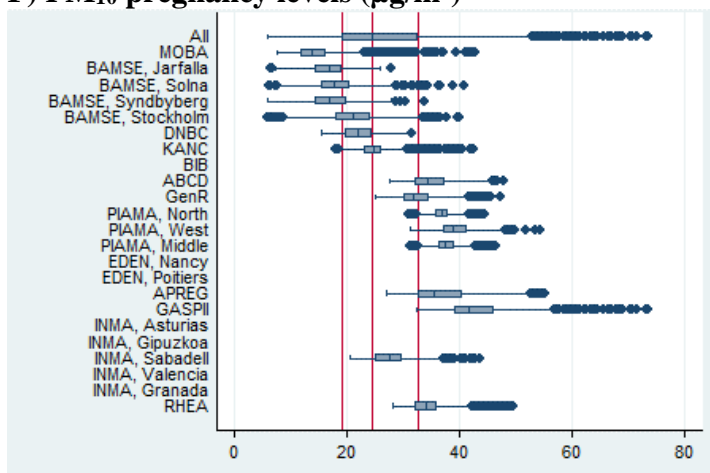
**D) NO<sub>2</sub> pregnancy levels (µg/m<sup>3</sup>)**



**E) PM<sub>2.5</sub> pregnancy levels (µg/m<sup>3</sup>)**

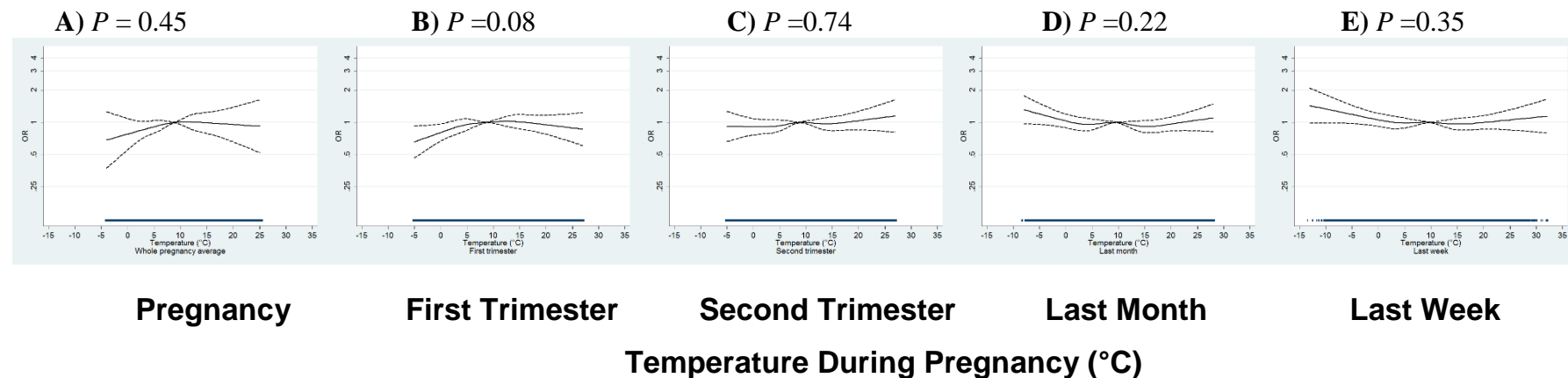


**F) PM<sub>10</sub> pregnancy levels (µg/m<sup>3</sup>)**



**Figure S V-2: Adjusted odds-ratios (OR) of preterm delivery associated with temperature at each time window (13 European birth cohorts, 1994-2010). Restricted cubic spline models adjusted for the covariates of model M2 (see Table V-2).**

- A) Whole pregnancy temperature average as estimated by a discrete time survival model;  
 B) Temperature averaged during the first trimester of pregnancy.  
 C) Temperature averaged during the second trimester of pregnancy.  
 D) Apparent effect of temperature monthly average on the risk of preterm birth the following month (as estimated by a discrete time survival model)  
 E) Apparent effect of temperature weekly average on the risk of preterm birth the following week (as estimated by a discrete time survival model).

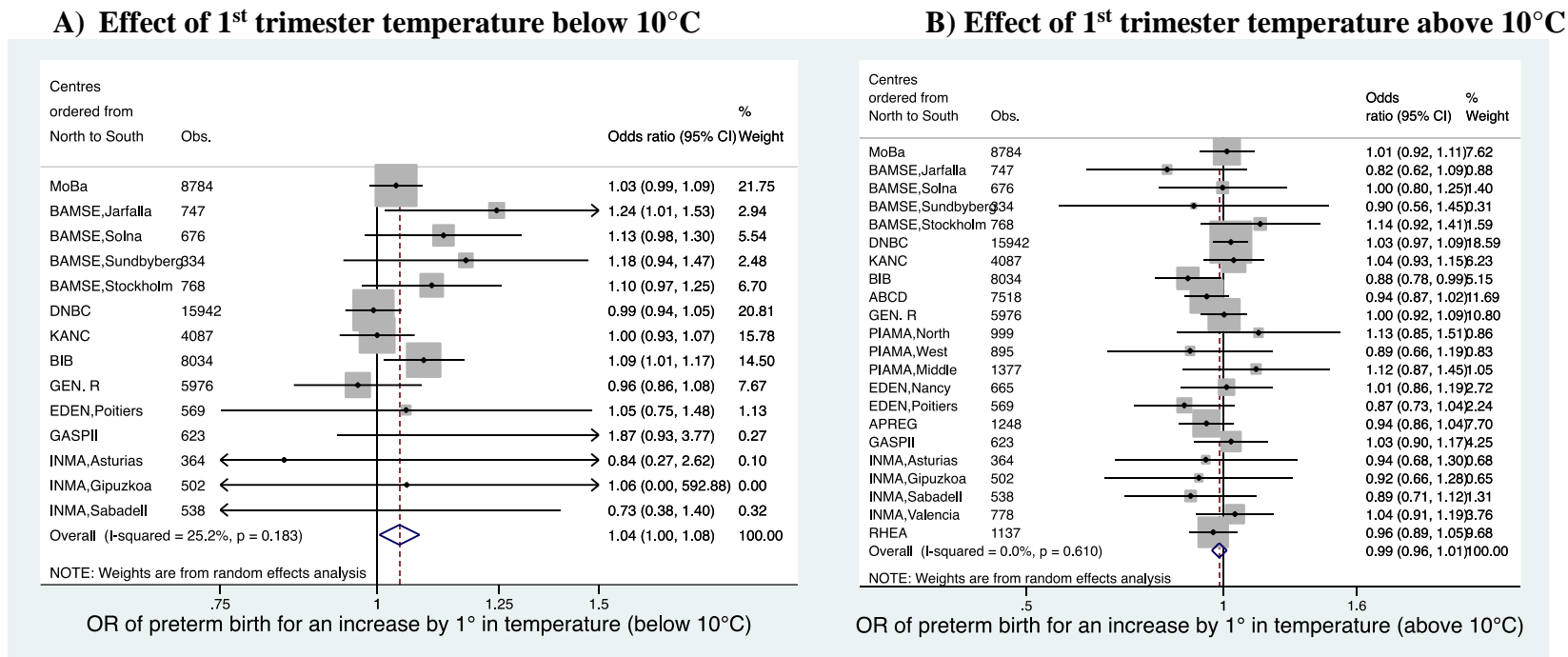


*P*-values are those testing the statistical significance of the cubic spline variables coding for temperature as a whole.

**Figure S V-3: Meta-analysis of the association between temperature first trimester level and preterm birth risk (13 European birth cohorts, 1994-2010).**

A) Adjusted odds-ratio (OR) of preterm delivery for each increase by 1°C in temperature first trimester average *below* 10°C.

B) Adjusted OR of preterm delivery for each increase by 1°C in temperature first trimester average *above* 10°C.



Models were adjusted for the factors listed in the footnote a) of Table V-2 (but center) and temperature was coded with a broken stick with a knot at 10°.



**Figure S V-4: Two estimates of the adjusted association between temperature pregnancy levels and the probability of preterm delivery (13 European birth cohorts, 1994-2010).**

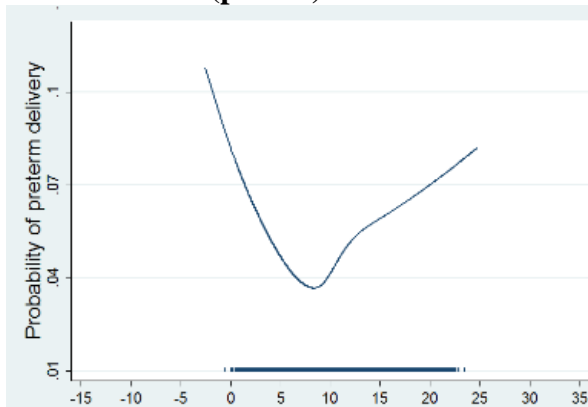
A) Logistic model in which temperature is averaged from conception until either the end of the 37<sup>th</sup> gestational week or birth, whichever comes first and

B) Discrete time Cox (survival) model with time-varying coding of temperature; this analysis was censored at 37 gestational weeks.

Both models were adjusted for the same factors (listed in the footnote *a* of Table V-2) and in both cases the temperature variable was coded with a restricted cubic spline model.

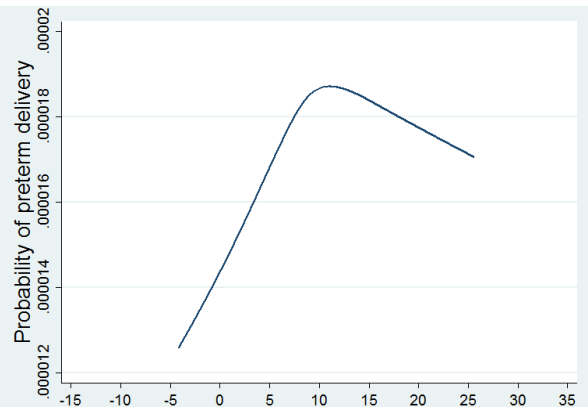
C) Distribution of temperature in term births (averaged until end of gestational week 37) and preterm births (averaged until birth).

**A) Logistic model ( $p < 5.10^{-3}$ )  
covariates ( $p = 0.45$ )**



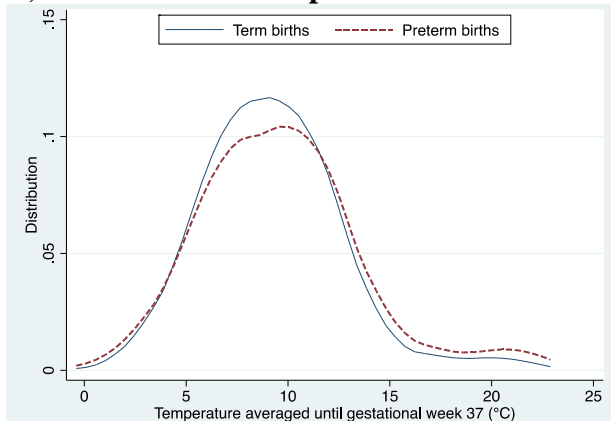
Temperature (°C) whole pregnancy average (censored at 37 gestational weeks)

**B) Survival model with time-varying**

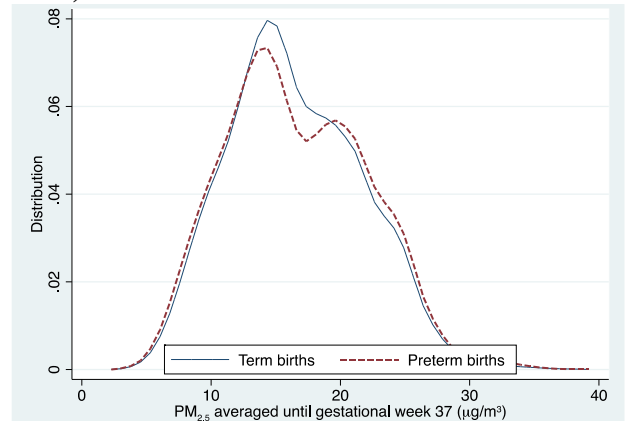


Temperature (°C) whole pregnancy average (censored at 37 gestational weeks), time-varying covariate

**C) Distribution of temperature**



**D) Distribution of PM<sub>2.5</sub> level**

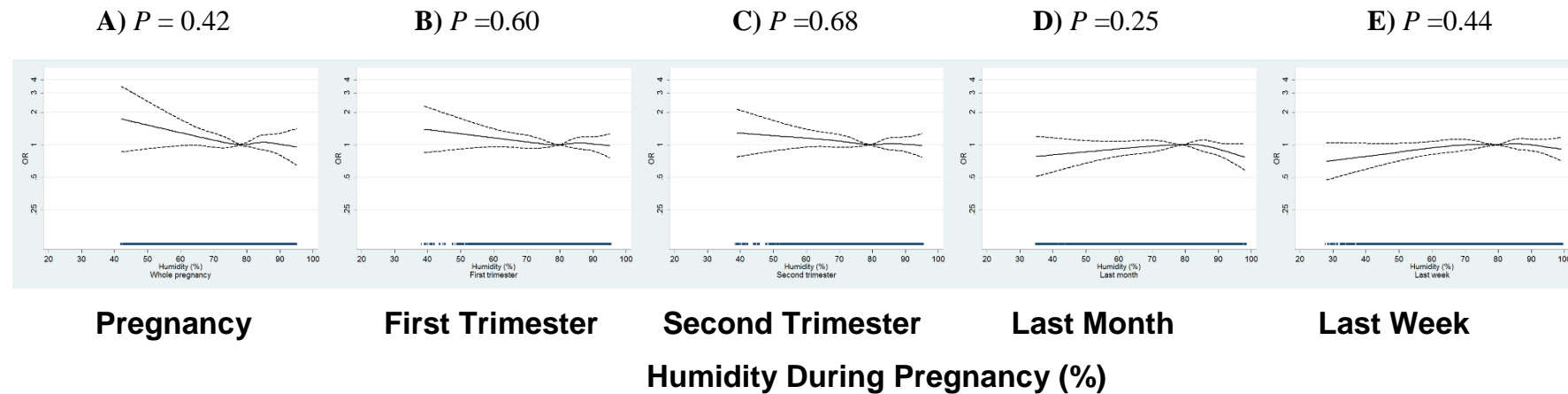


In the model of Figure S V-4-A, exposure is defined as the average of temperature between gestational week 1 and either gestational week 37 or birth, whichever came first, and the association with preterm birth risk is estimated through a logistic model. Since exposure is averaged over time windows of shorter durations in preterm compared to term births, and since temperature varies seasonally, the distribution of exposures in preterm births over-represents low and high temperatures, compared to the distribution of exposures in term births (Figure S V-4-C). As a result, preterm births are overrepresented at both extremes of temperatures, hence a bias for preterm birth risk to be higher for both low and high temperature, which corresponds to the pattern of Figure S V-4-A.

Using a discrete time Cox model with time-varying covariates allows to avoid this bias, and yields a different dose-response function, without much evidence of an effect of temperature whole pregnancy average (censored at 37 weeks) on preterm birth risk in this case (Figure S V-4-B). A similar pattern exists for PM<sub>2.5</sub> levels (Figure S V-4-D).

**Figure S V-5: Adjusted odds-ratios of preterm birth associated with humidity at each time window (13 European birth cohorts, 1994-2010).**

- A) Whole pregnancy humidity average (survival analysis);
- B) Humidity averaged during the first trimester of pregnancy.
- C) Humidity averaged during the second trimester of pregnancy.
- D) Apparent effect of humidity monthly average on the risk of preterm birth the following month (as estimated by a cox survival model)
- E) Apparent effect of humidity weekly average on the risk of preterm birth the following week (as estimated by a Cox survival model).



Restricted cubic spline models adjusted for the covariates of model M2 (see Table V-2, page 132).

$P$ -values are those testing the statistical significance of the cubic spline variables coding for humidity as a whole.

**Figure S V-6: Adjusted odds-ratios (OR) of preterm birth associated with atmospheric pressure at each time window (13 European birth cohorts, 1994-2010).**

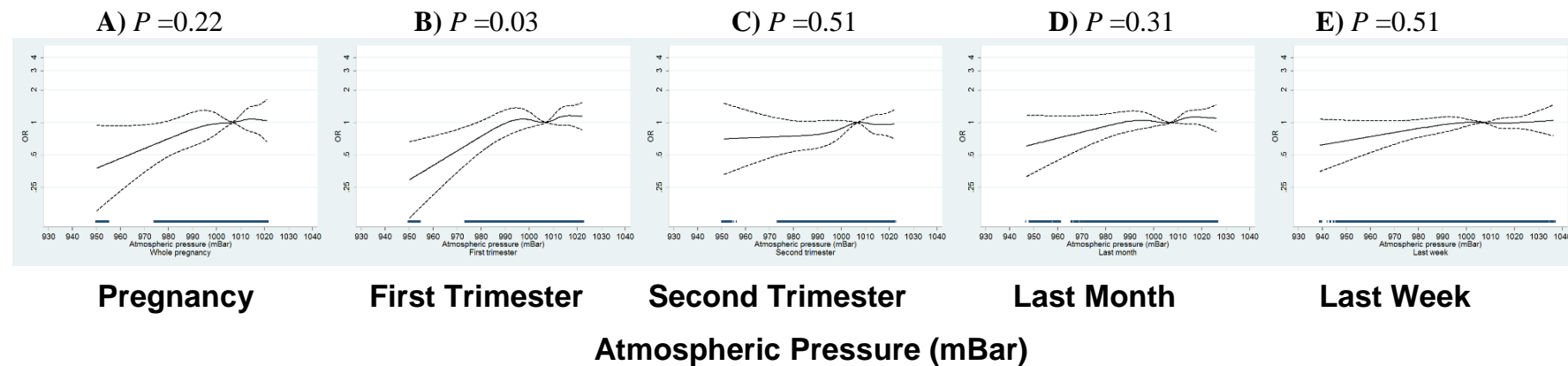
A) Whole pregnancy pressure average (survival analysis);

B) Pressure averaged during the first trimester of pregnancy.

C) Pressure averaged during the second trimester of pregnancy.

D) Apparent effect of pressure monthly average on the risk of preterm birth the following month (as estimated by a Cox survival model)

E) Apparent effect of pressure weekly average on the risk of preterm birth the following week (as estimated by a Cox survival model)

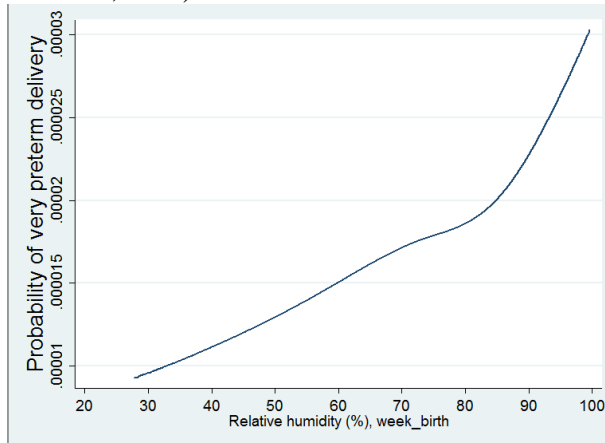


Restricted cubic spline models adjusted for the covariates of model M2 (see Table V-2, page 132).

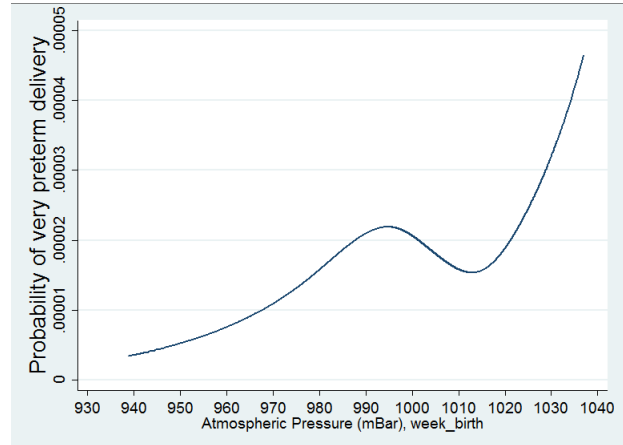
$P$ -values are those testing the statistical significance of the cubic spline variables coding for pressure as a whole.

**Figure S V-7: Adjusted odds-ratios (OR) of very preterm birth (before 32 completed gestational weeks) associated A) with humidity the previous week B) atmospheric pressure the previous week.**

**A) Humidity during the previous week**  
(restricted cubic spline coding  $P=0.35$ ;  
 $P$ -value associated with untransformed  
variable, 0.05)



**B) Atmospheric pressure the previous week**  
(restricted cubic spline coding,  $P=0.04$ )

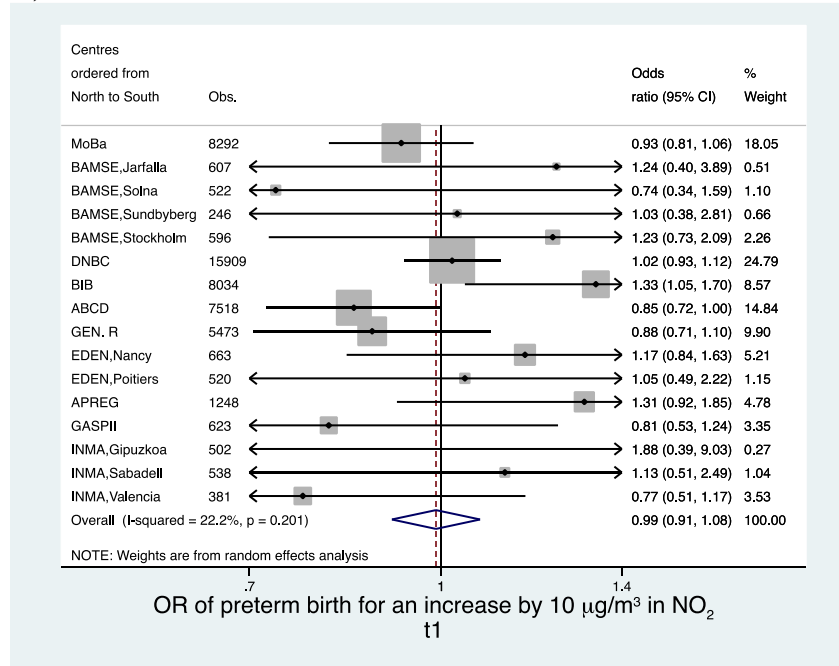


Discrete time survival models with restricted cubic spline coding of the meteorological variable, adjusted for the covariates of model M2 (see Table V-2, page 132)(13 European birth cohorts, 1994-2010).

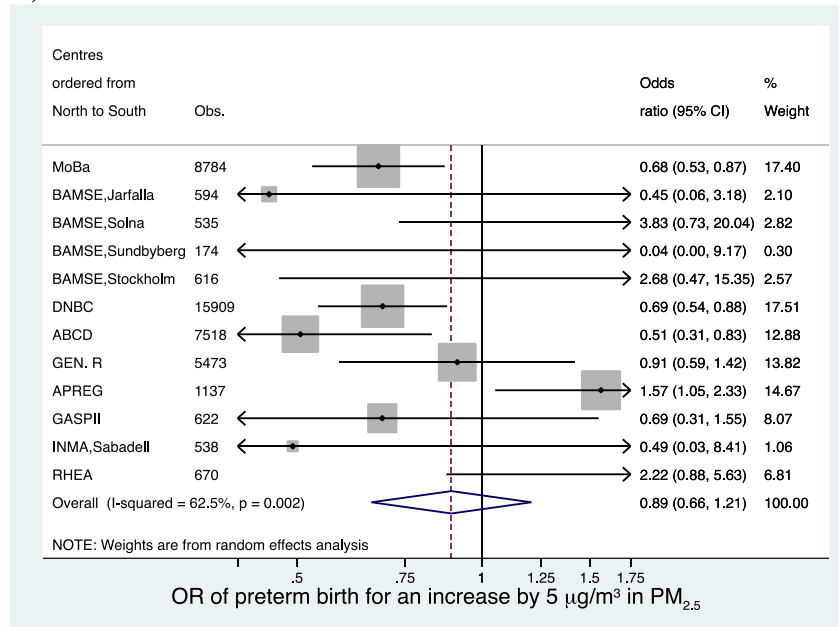
**Figure S V-8: Meta-analyses of the association between atmospheric pollutants and preterm birth risk (13 European birth cohorts, 1994-2010).**

Adjusted OR of preterm delivery for each increase **A)** by 10  $\mu\text{g}/\text{m}^3$  in  $\text{NO}_2$  first trimester average; **B)** by 5  $\mu\text{g}/\text{m}^3$  in  $\text{PM}_{2.5}$  first trimester average **C)** by 10  $\mu\text{g}/\text{m}^3$  in  $\text{PM}_{10}$  and **D)** by 10<sup>5</sup>/m in  $\text{PM}_{2.5}$  absorbance.

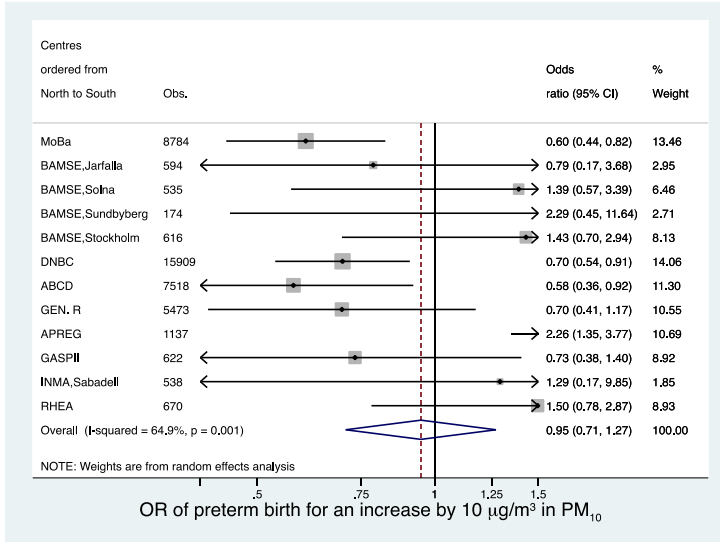
**A)  $\text{NO}_2$**



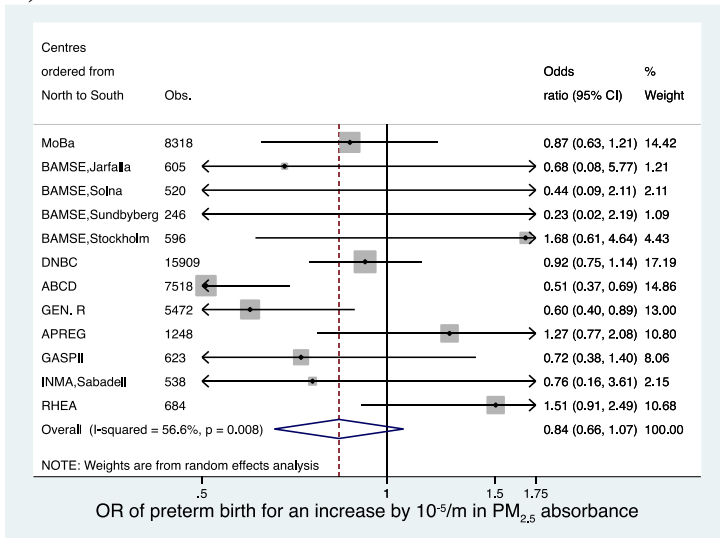
**B)  $\text{PM}_{2.5}$**



**C) PM<sub>10</sub>**



**D) PM<sub>2.5</sub> absorbance**





## Chapter VI: Discussion





## I. French summary

L'objectif de ce doctorat était d'étudier l'effet de la pollution atmosphérique sur la reproduction humaine. Nous nous sommes basés sur deux événements très peu étudiés -la fertilité et le cycle menstruel- et un étudié davantage, mais pour lequel très peu de facteurs de risques environnementaux ont été identifiés -naissances prématurées. Nous avons observé qu'une augmentation des niveaux de dioxyde d'azote (NO<sub>2</sub>) et de particules (PM<sub>10</sub>) dans le mois précédant un cycle menstruel était associée avec une augmentation de la durée de la phase folliculaire de ce cycle ; résultats qui doivent être répliqués par d'autres études. Nous n'avons pas observé d'association claire entre les niveaux de pollution atmosphérique et la fertilité ou la prématurité. Nos travaux ont reposé sur différents designs. Certains sont très peu utilisés, comme l'approche des durées en cours et de cohorte prévalente qui ont donné des résultats cohérents et ont été utilisés sur la même population. Nos travaux ont illustré qu'une partie de la littérature existante en faveur d'une association pourrait être sujette à un biais causé par des durées de fenêtres d'exposition différentes entre les enfants nés avant ou à terme. Dans l'ensemble, ce travail confirme la nécessité d'utiliser un modèle de survie avec variables dépendant du temps pour étudier le risque de naissance prématurité et appelle à poursuivre les recherches concernant des effets possibles des polluants atmosphériques sur le cycle menstruel et la fertilité, pour lesquels nos travaux font partie des premiers réalisés en population générale.

## II. Main findings

The objective of this thesis was to estimate the association between exposure to atmospheric pollution and various reproductive outcomes. In a study based on a subgroup of the OBSEervatory of Fecundity in France (OBSEFF), we observed that higher levels of NO<sub>2</sub> and PM<sub>10</sub> during the month before the start of a menstrual cycle were associated with longer follicular phase. This study is the first conducted on this topic. In the population recruited in OBSEFF study, we observed no clear short-term association between NO<sub>2</sub> and PM<sub>10</sub> levels and a marker of fecundity, in an original approach relying on two seldom used study designs in parallel. Our study was not strongly in favor of the association between atmospheric pollution and fecundity related outcomes suggested by previous reviews (Checa Vizcaíno et al., 2016; Frutos et al., 2015) but power was limited as shown by our relatively wide confidence intervals. In a last study, we did not observe associations between atmospheric pollution pregnancy exposures and preterm delivery. This study was based on pooled cohorts within the European Study of Cohorts for Air Pollution Effects (ESCAPE) project and decreased the level of proof regarding possible effects of particulate matter on preterm delivery, pointing possible bias relative to the choice of exposure windows and statistical method in previous studies. We observed an increased risk of preterm birth with higher atmospheric pressure during the first trimester of pregnancy and to some extent with temperature between -5°C and 10°C during the first trimester of pregnancy.

## III. Methodological issues

### 1. Confounding

Using data from cohorts instead of birth registers as done in many previous studies of preterm delivery risk (e.g. Huynh et al., 2006; Leem et al., 2006) allowed us to consider many potential confounding factors. In ESCAPE, pooling data from cohorts required a huge work of harmonization but allowed to reach a large sample size. As questionnaires differed between cohorts, some confounders could not be assessed homogeneously. For example, maternal active smoking was assessed at different trimesters during pregnancy and had to be imputed at second trimester for some cohorts. Alcohol consumption during pregnancy or detailed information for mode of delivery were sometimes unavailable and it was only possible to include these information in sensitivity analyses restricted to some cohorts.

In the OBSEFF study -and more importantly in the current duration setting where couples were recruited during the period of unprotected intercourse- we were not able to collect all the information on all potential confounders factors at the relevant time window (i.e. before the start of the period of unprotected intercourse for the current duration design). Couples involuntary infertile might change their behavior in order to have a child, for example stop smoking or go on a diet (behavior modification bias, Slama et al., 2006). Thus by collecting information only once the women are eligible, it is possible that, for example, the proportion of overweight women has been underestimated. Unfortunately, couples with long duration of unprotected intercourse might not be able to provide accurate information on behaviors at the start of the period of unprotected intercourse, and we were for example not able to adjust for frequency of intercourse at the start of the period of unprotected intercourse due to too many missing values.

## 2. Outcomes assessment

### *a. Menstrual cycle*

In our study of menstrual cycle characteristics, many women did not fill the booklet until the beginning of the next cycle (next menses), thus it was not always possible to determine if the last day of urinary collection was really the last day of their cycle. To deal with this issue, we considered that cycle and follicular lengths were censored and we used multiple imputation based on woman age (and follicular length if the cycle was ovulatory). This procedure probably did not impact strongly the results as adjusted linear analysis without imputation or a survival Cox analysis yielded similar conclusions.

### *b. Fecundity*

We defined time to pregnancy (and current duration of unprotected intercourse) in calendar months instead of number of menstrual cycles. We believe that, as not only pregnancy planners were recruited and as some of them had quite long duration of unprotected intercourse, answering in calendar time instead of number of menstrual cycles was easier for the participants. The questionnaire gave women the possibility to answer dates (birth of the last children, date at which each contraceptive method was stopped) or delays. Studies have reported that women with long time to pregnancy may have difficulties to retrospectively report time to pregnancy correctly (Cooney et al., 2009; Radin et al., 2015). Cooney et al. (2009)

observed that only 67% of women answered yes when asked 10 years after a pregnancy if they recalled what their time to pregnancy was, and 77% of them had an accuracy about 3 months. 10 years is quite long compared to the situation in our study as our participants were not using regularly any contraceptive method at recruitment and as durations above 3 years were censored. The Danish *Snart Gravid* Study (Radin et al., 2015) asked the same question to women during their first trimester of pregnancy and observed that on average the recall was accurate, but that women with a time to pregnancy above 2 months tended to underestimate it compared to women conceiving quicker; fecundability odds ratios associated with recent oral contraceptive use was biased away from the null by 10% when the retrospective time to pregnancy was used in the statistical analysis instead of the one recorded prospectively. We can assume from these results that (current) duration of unprotected intercourse was more accurately defined for pregnancy planners with a rather short duration of unprotected intercourse. Our results were not strongly modified by restricting our study population to pregnancy planners or couples with short current duration of unprotected intercourse (current duration design) or right-censored time to pregnancy (prevalent cohort design).

Additionally, questions about any previous pregnancy and the result of the last pregnancy test results were asked at three different time points in each questionnaire, which allowed us - after intensive data cleaning- to define as accurately as possible the date of the end of the previous pregnancy.

### *c. Preterm birth*

Whenever possible, we considered gestational duration based on conception date estimated from the last menstrual period (LMP) to define preterm birth. This choice was done because if the ultrasound method provides a good estimation of the due date, it is not reliable in epidemiological studies because if an exposure has an effect on the early embryo development (i.e. before the ultrasound measurement is performed), correcting the gestational age might introduce bias in the association to be identified (Basso and Olsen, 2007). Anyway, in the sensitivity analyses in which we restricted the population to those with both LMP based and ultrasound based date of conception, we observed that the associations between air pollution and preterm birth were not modified.

### 3. Exposure assessment

This thesis relied on fine-scale exposure models within generally urban area (LUR models developed in the scope of ESCAPE) or on a dispersion model with 1x1 km grid covering the whole country of France (OBSEFF study, exposure model developed within SYSCLAD project, Benmerad M, *in press*). As both exposure models provided estimates years after the period of interest, they were back-extrapolated and, in case of the LUR models which provided yearly estimated, seasonalized. Seasonalization and backextrapolation are procedures classically used in environmental epidemiology to study the association between air pollutants and reproductive outcomes (Lepeule et al., 2010; Pedersen et al., 2013a; Slama et al., 2007). If ESCAPE study was mostly conducted in urban areas, in is not the case for OBSEFF, for which using an exposure model covering the whole country allowed to include couples living in rural area, which would not be possible if only the nearest station was used. Including couples living in rural area allows to increase the exposure contrasts, but might also increase the potential for confounding and exposure misclassification (Pedersen et al., 2013b).

Results of environmental epidemiology studies can vary in function of the assessment of exposure; in particular exposure model used (Lepeule et al., 2010; Sellier et al., 2014), geocoding method (Jacquemin et al., 2013), seasonalization/backextrapolation (Chen et al., 2010) and accounting for residential mobility (Bell and Belanger, 2012) or space time activity (Ouidir et al., 2015; Setton et al., 2011).

#### *a. Back-extrapolation and seasonalization*

Many exposure models used in environmental epidemiology are available as yearly averages or are developed years after the period of interest. It is however essential to estimate exposures during the right time windows by taking into account both the year of exposure (back-extrapolation) and the period of the year corresponding to the exposure window if shorter than a year (seasonalization). In ESCAPE study, as some pollutants were not measured back in time, we had to study the correlations between the pollutants to design a method to back-extrapolate them (similarly to what was done previously by Slama et al., 2007). The approach that we used for back-extrapolation and seasonalization of ESCAPE LUR models make the assumption that one background monitoring station is enough to represent the temporal variation of the wider area covered by the LUR model. Regarding the OBSEFF study, as the study area was even wider, we considered several background monitoring station and used the

nearest (Gehring et al., 2011a). For this study, we combined exposures from the nearest station (at less than 100 km) with exposures in 2009-2010 estimated at home and station addresses from an exposure model with 1x1 km resolution. This procedure probably induce exposure misclassification, in particular because stations are attributed to women depending on the distance only and not taking into account characteristics of home and station locations.

Gulliver et al. (2016) compared a LUR model developed for Great Britain for the year 1991 to a LUR model developed for the year 2009 and back-extrapolated in 1991. The approach used was similar to the one we used in ESCAPE study but with difference correction instead of ratio correction<sup>13</sup>. The conclusion of this study was that the exposure levels predicted by the 1991 LUR model and the 2009 LUR model back-extrapolated in 1991 were similar in terms of exposure assessment. In Gothenbourg, Sweden, yearly exposures to NO<sub>x</sub> estimated in 1975, 1997 and 2005 by a dispersion model (hourly temporal resolution with spatial resolution of 50x50m) at the home of the Primary Prevention Study participants were compared to exposures from the 2009 dispersion model back-extrapolated with an undefined method. When going back farther in time, the correlations between the modelled and back-extrapolated NO<sub>x</sub> estimates decreased and the exposure levels of the study participants were underestimated (Molnar et al., 2015). The validation of the back-extrapolation assumption depends of the characteristics of the area where it is performed but seems to be appropriate in term of exposure estimates when going back for relatively short duration (5-10 years) following the results from the studies in Great Britain and Gothenbourg (Gulliver et al., 2016; Molnar et al., 2015).

Using or not using back-extrapolated or seasonalized exposures (Slama et al., 2007; Wu et al., 2011) and the choice of the backextrapolation method (Chen et al., 2010) -which include finding a solution when the monitoring sites did not measure the pollutant of interest, as we did for ESCAPE study and as previously done by Slama et al. (2007)- may modify the association between exposure and outcome. Our assumption was that the possible error introduced by assuming that monitoring stations describe appropriately the temporal variations of their surroundings and by using temporal component estimated from other pollutants was smaller than the error induced by using the temporally unadjusted exposure.

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<sup>13</sup> [http://www.escapeproject.eu/manuals/Procedure\\_for\\_extrapolation\\_back\\_in\\_time.pdf](http://www.escapeproject.eu/manuals/Procedure_for_extrapolation_back_in_time.pdf)

### *b. Geocoding*

Several methods can be used to geocode addresses (i.e. turning a string address into geographical coordinates). When an exposure model with fine spatial resolution is used, the choice of the geocoding method can impact the association between exposure and health (Jacquemin et al., 2013). In ESCAPE study, geocoding the address(es) of each cohort participant was not performed centrally and methods used probably differed. The impact this might have on the estimated association is unknown. In a comparison of several geocoding methods done by Jacquemin *and colleagues* (2013), when the building matching method was used to geocode addresses, the associations observed between air pollutants (NO<sub>2</sub> and PM<sub>10</sub>) and forced expiratory volume in 1 second or forced vital capacity were stronger than when spatial interpolation methods were used. In the OBSEFF study, addresses for women included in current duration design were geocoded by a contractor with an unspecified method while women ineligible in current duration study but eligible in prevalent cohort were geocoded automatically using the IGN (French national geographic institute) addresses database (geocoding at address plate or if not possible with spatial interpolation) or, if not found in the database, manually with building matching (cadastral register). As the chemical-transport exposure model used in OBSEFF study had a 1×1 km resolution (contrarily to 10m×10m in Jacquemin et al), the geocoding method probably did not impact our results.

### *c. Residential mobility*

We tried whenever possible to limit exposure misclassification by taking into account moving. For the OBSEFF study, we considered exposure at the recruitment address (Q0) for women eligible in the current duration analysis and at first follow-up otherwise; but did a sensitivity analysis by considering only women declaring that they did not move since the beginning of the time of unprotected intercourse (current duration design). Previous address was unknown. This issue did not concern the prevalent cohort design except for women who moved very shortly before the entry in the study as addresses were recorded at the time when the exposure were estimated. For the menstrual cycle study, addresses at recruitment interview (Q0) were used. Samples were collected in median 1.0 month (75<sup>th</sup> percentile: 1.7 month, maximum: 6 months) after recruitment Q0 and information about women moving between recruitment and sampling is unknown. However, as the delay is short, it is unlikely that a high number of the participating women moved during this period of time. In ESCAPE study, we took into account moving during pregnancy by weighting the exposures at each address in



function of the time spent at each address for the cohorts that provided detailed address history (BAMSE, DNBC, Generation R, EDEN and APREG).

Generally, it is important to take into account moving as according to countries, 9%-32% of mother reported to move during pregnancy (Bell and Belanger, 2012). Birth cohorts should record addresses during pregnancy and the follow-up of the child, even if the true impact of not taking account moving during pregnancy on the association with birth outcome is unknown. Moreover, articles about exposure misclassification due do moving failed sometimes to consider exposure at the right exposure windows (for example, weighted annual average were used for  $PM_{10}$  exposure by Hodgson et al., 2015) or use models of exposure with various spatial resolutions, which will be related to the exposure misclassification. Although the impact of exposure misclassification due to moving is usually assumed to be limited, it must be noted that it depends in fact highly of the exposure model used. Fell et al. (2004) observed in a Canadian study that among the 12% of women who moved during pregnancy, 62% stayed in the same municipality. In their review of the research on residential mobility during pregnancy, Bell and Belanger (2012) noticed that most studies reporting the distance moved had a median distance moved inferior than 10 km, with most mothers staying in the same general area. Using a nearest station model (with a poor spatial distribution) may not modify much the estimated exposure in this population, but using dispersion models with 10mx10m spatial resolution may entail stronger changes on exposure estimates, and possibly on the dose response function. The expected bias on the dose-response function is attenuation bias (Bell and Belanger, 2012; Setton et al., 2011), assuming that the exposure level at the new address is independent of that of the previous address, which can be debated.

#### *d. Time space activity*

Incorporating time-space activity in air pollution exposure assessment is another classical issue in environmental epidemiology. In a recent study, Ouidir et al. (2015) showed that the use of Global Positioning System (GPS) data cleaned using diaries did not substantially modify the exposure assessment to  $PM_{2.5}$  compared to an exposure estimated at the home address only in a population of urban pregnant women from Grenoble, France. The impact on  $NO_2$  exposure estimates (a pollutant with stronger spatial variations than  $PM_{2.5}$ ) was somewhat larger; however in the first trimester of pregnancy -the one during which pregnant women spent least time at home, with a median of 15h per day at home in this French population- the correlations

between exposures to NO<sub>2</sub> and PM<sub>2.5</sub> estimated at the home address and with clean GPS data remained high ( $r > 0.98$ ). In this study, exposure to NO<sub>2</sub> and PM<sub>2.5</sub> were estimated using a dispersion model with a 10m x 10m grid, seasonalized with one background monitoring station. Pregnant women are a specific population, and if we can assume from the analysis performed by Ouidir et al. (2015) that the exposure estimates of ESCAPE study might not have been modified by incorporating time-space activity, it is not generalizable to the OBSEFF population of non-pregnant women. Among the 867 women with defined duration of unprotected intercourse, the median time at home per day was 19h during the week before questionnaire Q0 (which is high compared to the pregnant women from Grenoble, but less accurate as the OBSEFF questionnaire was asked retrospectively while the pregnant women filled a diary each day and carried GPS). With the use of an exposure model with a spatial resolution of 1 x 1 km, it is unlikely that the exposure estimates would have been modified to a very large extent had we considered time-space activity in the OBSEFF study.

The impact of not taking into account daily mobility is estimated to correspond to attenuation bias. A simulation conducted by Setton et al. (2011) highlighted that the higher is the resolution of the exposure model, the larger the attenuation bias is in the estimated dose response functions.

#### 4. Study design and statistical methods

##### *a. Menstrual cycle study*

Very few studies considered menstrual cycle characteristics together with environmental exposures and a study design allowing to define day of ovulation. Indeed, many studies used questionnaires and only have information such as cycle and menses lengths (Cooper et al., 2005; Lawson et al., 2011; Toft et al., 2008). In studies that were able to define lengths of follicular and luteal phases, environmental exposures that have been considered include active smoking (Windham et al., 1999), chlorination by-products (Windham et al., 2003), organochlorine compounds (Windham et al., 2005) and persistent organohalogen and metals (Wainman et al., 2016). Our study, by reporting the association observed with cycle, follicular phase and luteal phase lengths and air pollutants is quite innovative.

### *b. Fecundity studies*

Several designs can be used to study time to pregnancy, which is a marker of fecundity. They are defined depending on when the women (or couples) are recruited compared to the onset and end of the period of unprotected intercourse. The characteristics and biases of each method have been summarized by several articles (Slama et al., 2006, 2014; Weinberg et al., 1994) and are described in Table VI-1 (characteristics) and Table VI-2 (biases). To summarize, the pregnancy-based approaches (or based on the last pregnancy, or historically retrospective) are the easiest methods to set up. Their major flaw is their inability to recruit infertile couples, leading to a possibility that the association between exposure and fecundity is biased towards the null as for example suspected by Sallmén *et colleagues* for paternal exposure to lead (Sallmén et al., 2000). The current duration approach, one of the two designs used in this thesis to study the association between atmospheric pollution and fecundity, is the second easiest design to set up as no follow-up of couple is needed. It has the advantage to include infertile couples, yet the eligibility rate is very low (only 6% of the women that answered the eligibility questionnaire participated in OBSEFF study; Slama et al., 2012) and make the assumption that the initiation times are stable over time, which can be debated, as observed in our follow-up data, or in five European countries (Basso et al., 1995). The prevalent cohort is the second approach used during this thesis. It necessitates to recruit the same population than the one included in the current duration approach and then to follow the identified couples. Unlike pregnancy based studies and current duration approach, this approach is not prone to bias due to time trends in exposure, but as both previous methods, recall error or digit preference can occur. The last possible approach is the incident cohort, consisting to identify couples before they stop contraception and to follow them up. The major difficulty with this approach is to identify those couples and to not over-represent the couples that planned their pregnancy several months ahead (called “super pregnancy planners”, Slama et al., 2004) as they can be quite specific in term of exposures and socio-economical characteristics (Slama et al., 2004).

It is difficult to conclude on one design that would be the best to study time to pregnancy. Pregnancy-based designs are probably the approaches that are the more prone to biases. The incident cohort is very interesting if the issue of the “super pregnancy planners” can be avoided. The combination of current duration and prevalent cohort designs as we did in OBSEFF is interesting. We observed that the directions of the associations between the confounders and the probability of pregnancy were quite consistent in the two designs, except for passive smoking and education level. Regarding exposure to atmospheric pollution, PM<sub>10</sub> was

associated with a decreased fecundity level in the current duration design (exposure window corresponding to 70 days before the beginning of the period of unprotected intercourse, TR per  $10 \mu\text{g}/\text{m}^3$  increase: 1.29, 95% CI 0.97;1.70) while in the prevalent cohort design, only a trend for decreased fecundity level was observed (exposure window corresponding to 70 days before the entry in the survival analysis, HR of pregnancy per  $10 \mu\text{g}/\text{m}^3$  increase: 0.69, 95% CI 0.47;1.12). We first assumed that the results of the current duration analyses were due to the fact that air pollutants levels decreased during the study period: as exposure window was the 70 days before the beginning of the current duration of unprotected intercourse, longer current durations of unprotected intercourse were by design assigned to higher level of exposure compared to shorter current durations of unprotected intercourse, while in the prevalent cohort, the exposure window was the 70 days before the entry in the survival model. However, when purely spatial exposures were used, the time ratios associated with exposure levels were not weakened. This surprising result may be due to another bias in our current duration analysis: in OBSEFF study, couples have been recruited during a few winter months. By using the exposures based on the adapted CHIMERE model estimated at the right exposure windows but transposed to 2009, we removed the long term temporal trends; but as shorter current duration of unprotected intercourse began in late autumn or winter, they have higher exposure levels than longer current duration of unprotected intercourse which began at any time through the year. Thus the seasonal trends may induce a bias in the estimated analysis when the exposure windows considered last less than a year.

**Table VI-1: Summary of the features of the study designs for human fecundity**

	<b>Designs</b>			
	<b>Current duration study</b>	<b>Prevalent cohort</b>	<b>Incident cohort</b>	<b>Pregnancy-based design</b>
<b>Principle</b>	Identify couples not using any contraceptive methods	Identify couples not using any contraceptive methods and follow them up	Identify couples before they stop contraception	Identify couples who already have a child (or children)
<b>Recruitment</b>	During the period of unprotected intercourse	During the period of unprotected intercourse	Before the period of unprotected intercourse	After the period of unprotected intercourse
<b>Follow-up necessary?</b>	No	Yes	Yes	No
<b>Outcome observed</b>	Current duration of unprotected intercourse (can end by a pregnancy but also other reasons)	Time to pregnancy (or censor or competing event)	Time to pregnancy (or censor or competing event)	Time to pregnancy
<b>Possible statistical method</b>	Accelerated failure time model (Keiding et al., 2012)	Survival model with delayed entry	Survival model	Survival model
<b>Censoring and consideration of competitive risk</b>	Censoring possible by excluding couples with long current duration of unprotected intercourse or fertility treatment. Not possible to know how the current duration of unprotected intercourse will end.	Censoring possible at the fertility treatment onset or after a definite numbers of months without conceiving. Interrupting the period of unprotected intercourse by another outcome than pregnancy should preferably treated as competitive event.	Censoring possible at the fertility treatment onset or after a definite numbers of months without conceiving. Interrupting the period of unprotected intercourse by another outcome than pregnancy should preferably treated as competitive event.	Censoring possible at the fertility treatment onset or after a definite numbers of months without conceiving.
<b>Inclusion of non-pregnancy planners?</b>	Yes	Yes	Yes, but pregnancy planners are easier to identify in this setting.	Yes, but unplanned pregnancies might be described retrospectively as planned
<b>Inclusion of pregnancies due to contraception failure?</b>	No	No	Difficult but possible	Yes, but might retrospectively be described as planned pregnancy.
<b>Inclusion of infertile couples that will never succeed in conceiving?</b>	Yes	Yes	Yes	Generally not, but feasible (e.g. Slama et al., 2008)

**Table VI-2: Possible bias in the study designs used to study fecundity and possible solutions.**

Source of bias and description	Current duration study	Prevalent cohort	Incident cohort	Pregnancy-based	Possible Solution
<b>Study design</b>					
Infertile couples not included				X	Try to identify unsuccessful attempts
Over representation of “super pregnancy planners”			X		Follow-up the screened population to include also unplanned and contraception failure pregnancies.
Stability over time of initiation times	The system must be in a stationary state			Truncation of short/long TTPs at the beginning/end of the study period	Define the study period with respect to the date of the beginning (and not the end) of the period of unprotected intercourse. Weighting approach possible for current duration design.
Pregnancy planning bias 1: Exclusion of unplanned or mistimed pregnancies	Include non-planner.	Include non-planner.			Ascertain exposures for non-planners as well and conduct sensitivity analyses
<b>Outcome assessment</b>					
Pregnancy planning bias 2: Unplanned pregnancy retrospectively described as planned (wantedness bias)				X	Conduct sensitivity analysis excluding conceptions during first cycle and define inclusion criteria on contraceptive use rather than pregnancy wish.
Pregnancy planning bias 3: Non pregnancy planner couples excluded			X	X	Try to include couples not planning to become pregnant and do a sensitivity analysis.
Pregnancy recognition bias: Delay in recognition of pregnancy is similar across exposure categories	record when pregnancy was recognized and follow-up	Provide pregnancy test kits	Provide pregnancy test kits	record when pregnancy was recognized	Record when and how pregnancy was recognized and restrict analyses to pregnancies leading to a livebirth.
Use of oral contraception may vary with exposure and might be associated with decreased fecundability in the first cycles	X	X	X	X	Ask the last contraceptive methods used and in particular if couples used abstinence after discontinuation of pill. Pill use can be incorporated in the model as a time-varying covariate.
In a retrospective setting, couples may recall time to pregnancy or current duration of unprotected intercourse with some error	X	X		Underestimation possible	Focus on pregnancies leading to a livebirth, for which recall may be better; careful questionnaire design
Differences in desired or achieved family size	X	X	X	X	?
<b>Exposure assessment</b>					
Bias due to time trends in exposure and TTP	X			X	Simulate by using external data on the time trends in exposure
Exposure is assessed during or after pregnancy instead of at the start of the period of unprotected intercourse.		X	X	X	Assess exposure at the start of the period of unprotected intercourse
Behavior modification bias and assessing exposure during or after pregnancy instead of during	X	X	X	X	
<b>Statistical analysis</b>					
Proportional hazard hypothesis not verified		X	X	X	Test for different effects of exposure during months 1–3 and 4–12 (or other cutoffs) of the pregnancy attempt.
Medical intervention bias	Censor (corresponding to exclusion)	Censor	Censor	Censor	Try to assess if censoring is informative
Bias due to differential persistence in trying	Compare frequency of treatments			X	

Table adapted from Slama et al., 2006, 2014; Weinberg et al., 1994

*c. Preterm delivery study*

Many studies have dealt with the association between atmospheric pollution and preterm birth risk using various designs: birth or pregnancy cohorts (e.g. in PIAMA cohort, Netherland, Gehring et al., 2011a; or INMA cohort, Spain, Llop et al., 2010), birth registers (e.g. in Czech Republic, Bobak, 2000; or in USA, Chang et al., 2015) and time-series analyses of birth registers (e.g. in Roma, Italy, Schifano et al., 2013, or in London, United Kingdom, Lee et al., 2008). Various exposure windows have been tested. Some have been defined from a starting date (i.e. date of conception or last menstrual period: whole pregnancy censored at 37 weeks, each trimester, each month of pregnancy, each week) or from the end date (i.e. birth: whole pregnancy uncensored, lasts weeks, lasts months). Very few studies considered the fact that exposures defined in exposures windows ending at birth (whole pregnancy, 3<sup>rd</sup> trimester) have shorter length for preterm versus term births. Indeed, preterm births have higher rates of extreme exposure values even in the absence of a causal effect of exposure on preterm delivery risk as the variability of air pollution average increases for smaller exposure windows. Some studies took this issue into account by truncating exposures at the last day of gestational week 36, corresponding to day 259 after LMP (e.g. 3<sup>rd</sup> trimester is truncated in Gehring et al., 2011b) or by not considering these exposure windows (e.g. Lee et al., 2013). In ESCAPE study, we observed that truncating exposure at 259 days was not enough to remove bias in dose response function when a logistic regression was used to study the association between temperature during pregnancy and preterm birth risk: in this study, a U-shape relation between temperature whole pregnancy average (censored at 37 weeks of gestation) and preterm birth appeared but it was not present when a survival model was used (supplemental material of the ESCAPE preterm paper, see Figure S V-4, page 143). Temperature, as atmospheric pollution, is an exposure varying other time with a strong seasonal pattern: studies using logistic regression to estimate the association between atmospheric pollutants and preterm birth may be impacted too. Yet, logistic regression is the most used statistical method to study the association between preterm delivery and environmental exposures to meteorological parameters and atmospheric pollution (for the example of studies conducted on PM<sub>2.5</sub>, see the example of the studies reviewed by Sun et al., 2015, in Table I-4, page 27 and discussed later in this paragraph). Truncating the exposure window earlier is possible but prevent studying the effect of the exposures occurring after gestational weeks 28-30; thus a statistical method allowing to incorporate time-varying variables is needed to appropriately consider the exposure windows if its length depends on the case status or for short term exposure before birth (last week, last month) to ensure comparison of the exposures at the same time for preterm and term births.

This issue concerns exposure windows during whole pregnancy and third trimester of pregnancy, but also during last months and last weeks of pregnancy. Indeed, by using logistic regression to estimate the association between preterm delivery and exposure during the last month of pregnancy, a preterm child born on day 225 (gestational week 32, exposure averaged over days 194-224) will be compared to a term child born on day 295 (gestational week 42, exposure averaged over days 264-294). Using a model incorporating time-varying variables implies that the exposure during days 194-224 of children born at gestational week 32 (“cases”) are compared to exposure at a similar gestational age for all at risk children (“controls”: term children but also preterm children born after week 32). This issue has been known for years (see Slama et al., 2008, or in the context of urinary tract infections and preterm birth, S O’Neill et al., 2003), but studies ignoring it have still been recently published (e.g. Qian et al., 2016; Zhao et al., 2015). One study had also been first published with an inadequate statistical model -logistic regression- and re-analyzed later -conditional logistic regression matched for gestational age- (Wu et al., 2009, 2011). In this case, the impact of whole pregnancy exposure to NO<sub>x</sub> and PM<sub>2.5</sub> estimated by a dispersion model during pregnancy was not very different between the two study designs with increased risk of preterm delivery observed with higher level of pollutants. This study did not consider meteorological factors and was not adjusted for all possible confounders, such as maternal smoking (not available in the hospital database) or newborn sex (available but not included in analysis because not considered as an *a priori* confounder).

As highlighted previously, many publications used inappropriate statistical methods. To illustrate this, let us consider the example of the studies included in one of the most recent meta-analysis conducted on preterm birth and fine particulate matter (Sun et al., 2015). This review included 18 studies published before December 2014 with results on the association between preterm delivery and PM<sub>2.5</sub>; it reported significant heterogeneity in studies. The study characteristics are given in Table I-4, page 27. Only 4 studies out of 18 considered meteorological parameters as a source of confounding (Chang et al., 2015; Jalaludin et al., 2007; Pereira et al., 2014b, 2014a). Among the 18 studies, 14 used logistic regression or equivalent models for their analysis (Brauer et al., 2008; Fleischer et al., 2014; Gehring et al., 2011a; Gray et al., 2014; Ha et al., 2014; Hannam et al., 2014; Hyder et al., 2014; Jalaludin et al., 2007; Kloog et al., 2012; Lee et al., 2013; Ritz et al., 2007; Rudra et al., 2011; Wilhelm and Ritz, 2005; Wu et al., 2009; but Lee et al. (2008) only considered exposures during first trimester) and only one a discrete-time survival model with time-varying exposures (Chang et al., 2015).



Other studies used a case-controls design: one appropriately matched exposures of cases and control (Huynh et al., 2006) while the last two publications only mentioned that they truncated the exposure at 36 weeks for third trimester and whole pregnancy exposure windows (Pereira et al., 2014b, 2014a), which, in our experience (Giorgis-Allemand, L et al., *In press*) is not enough to prevent bias. Sun et al. estimated a meta-analytical OR of preterm delivery associated with exposure to PM<sub>2.5</sub> during the whole pregnancy of 1.13 (95% CI 1.03-1.24, p-value < 0.05, 13 studies) per each increase by 10 µg/m<sup>3</sup>, 1.08 (0.92-1.24, pvalue>0.10, 10 studies) for 1<sup>st</sup> trimester exposure, 1.09 (0.82-1.44, pvalue>0.10, 5 studies) for 2<sup>nd</sup> trimester exposure, 1.08 (0.99-1.17, 0.05<pvalue<0.10, 9 studies) for 3<sup>rd</sup> trimester exposure, 1.10 (0.92-1.30, pvalue>0.10, 3 studies) first month of pregnancy and 1.01 (0.86-1.19, pvalue>0.10, 6 studies) for exposure during the month before birth. Associations were thus strongest with the whole pregnancy and the 3<sup>rd</sup> trimester exposures, which are two exposures windows that might be impacted by the above mentioned bias. The two studies using an appropriate definition of exposure are Chang et al. (2015) with a survival model and Huynh et al. (2006) with a matched case-control design comparing exposures for cases and controls at the same gestational ages. Both Chang et al. (2015) and Huynh et al. (2006) observed that higher levels of PM<sub>2.5</sub> during pregnancy were associated with increased risk of preterm delivery (OR, 95% CI per increase by 10 µg/m<sup>3</sup>, re-estimated by Sun et al. during pregnancy: 1.10 [1.00;1.21] for Chang et al. and 1.17 [1.08;1.28] for Huynh et al.) and their point estimates were weaker than five other studies included in the meta-analysis (out of the eleven other studies with results for whole pregnancy exposure included in the meta-analysis). Regarding the third trimester results, the OR estimated by Chang et al and re-estimated by Sun et al. was 1.00 (95% CI, 1.98;1.02) per 10 µg/m<sup>3</sup> increment in PM<sub>2.5</sub>.

From our experience with ESCAPE study, results from studies observing an association between preterm delivery and environmental exposure during whole pregnancy or third trimester of pregnancy although not using survival model with time-varying exposure cannot be trusted. It is suggested that authors studying preterm delivery carefully describe in their methodological section how they estimated exposures during pregnancy and third trimester and the statistical method they used. Our study, following others, calls for not relying on logistic regression for these exposure windows outside the setting of matched case-controls design.

Contrary to Chang et al (2015) and Huynh et al (2006) we did not observe an association between exposure to PM<sub>2.5</sub> during pregnancy and preterm birth risk (OR and 95% CI per increase by 10 µg/m<sup>3</sup>: 0.92, 0.76;1.08). The plausibility of this literature will be discussed later in this chapter in part IV.3, page 171.

#### IV. Plausibility of the findings

##### 1. Do air pollutants levels influence menstrual cycle length?

Atmospheric pollution levels were associated with increased duration of follicular phase. The pathway whereby atmospheric pollution could influence menstrual cycle characteristics is unknown. Veras et al. observed that mice exposed to non-filtered PM<sub>2.5</sub> had longer estrus (“heat”) and longer oestrous cycles than mice in chambers filtered for PM<sub>2.5</sub> (Veras et al., 2009). Menstrual cycles in humans and oestrous cycles in mice are not comparable, but Veras et al suggested that the induced persistent estrus may reflect impaired ovulation and changes in circulating ovarian hormonal levels.

Female active smoking has been associated with shorter duration of follicular phase (Liu et al., 2004; Windham et al., 1999). In our studies, there was a trend for a shortened follicular phase length with active smoking (n=162,  $\beta=-.69$ , 95% CI -2.01;0.70<sup>14</sup>), and active smoking was not clearly associated with luteal phase length (n=146,  $\beta=-.30$ , 95% CI: -1.50;0.91<sup>15</sup>) or cycle length (n=168,  $\beta=-.20$ , 95% CI -3.03;2.64<sup>16</sup>). Liu et al. (2004) explained that active smoking might accelerate follicular maturation by affecting Follicular Stimulating Hormone (FSH) production: the FSH drive increased and, by stimulating ovarian follicles development, the follicular development may be truncated. In the case of air pollution, the ovarian follicles may be not stimulated enough or ovulation be postponed. Watanabe and Oonuki (1999) additionally observed that exposure to diesel exhaust decreased FSH and LH serum levels in male rats (which, with testosterone, are hormones controlling testicular function in male; Ramaswamy and Weinbauer, 2015). This would be coherent with longer follicular maturation if the level of hormones secreted by pituitary gland are also decreased in females. Our study did not provide any information on a possible mechanism but, it as it is the first study on humans and as the study performed on mice give plausibility for an effect of air pollution on menstrual cycle characteristics, it would be interesting to replicate the analyses in another population or to study if air pollution is associated with anovulatory cycles or hormonal circulating levels.

<sup>14</sup> Linear regression adjusted on woman age (<30;30-34;35-39;40-45 years), body mass index (<25,  $\geq$ 25 kg/m<sup>2</sup>, 3 missing values imputed in the lowest category), age at menarche (8-12;12-18 years, 3 missing values imputed in the higher category), parity (nulliparous/parous), alcohol consumption in the week before inclusion (yes/no), caffeine consumption in the week before inclusion (yes/no), active smoking at inclusion (yes/no), passive smoking at inclusion (yes/no), education level (3 categories) and professional activity (yes/no)

<sup>15</sup> See note 12 for regression model and adjustment factors. Missing values for luteal phase (n=29) are imputed 1,000 times using age and follicular length.

<sup>16</sup> See note 12 for regression model and adjustment factors. Missing values for cycle (n=41) are imputed 1000 times using age and follicular length if the cycle is ovulatory, age only if anovulatory.

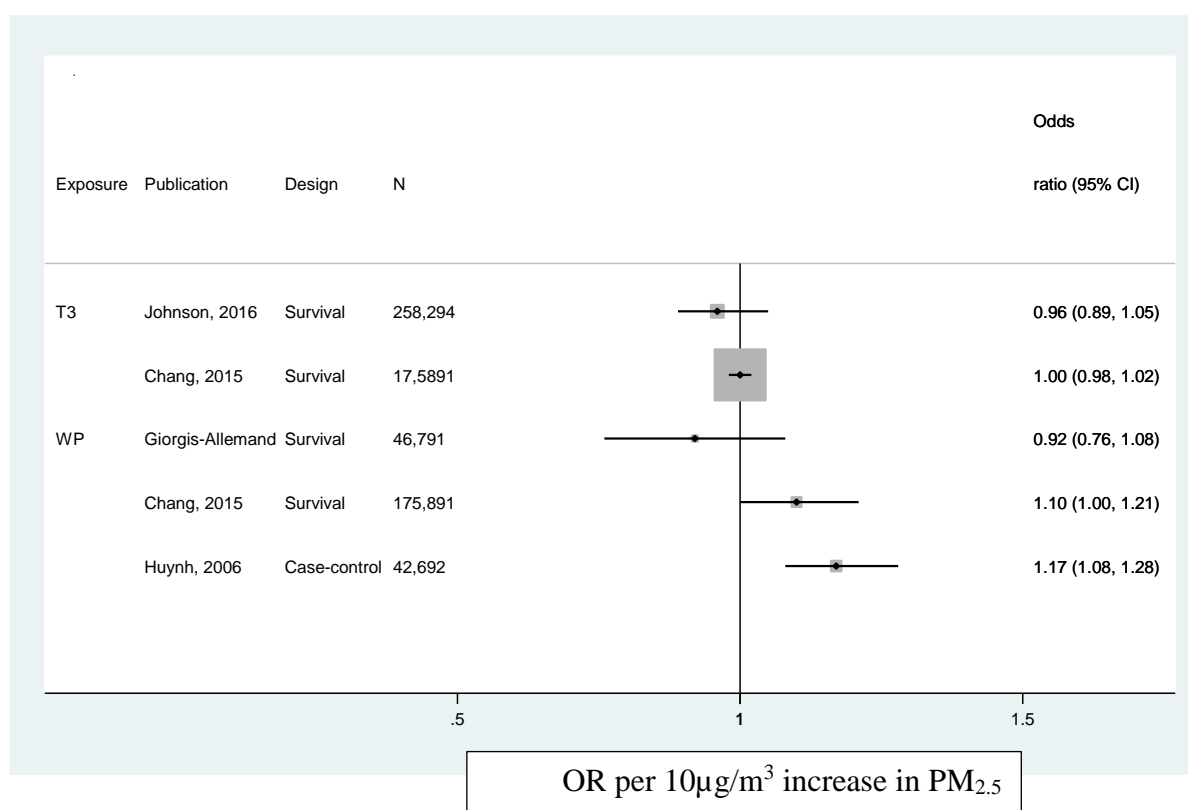
## 2. Do air pollutants levels influence fecundity?

By using two design in parallel in the same (general) population we did not observe a clear association between air pollution and reduced fecundity, studied using time to pregnancy as a marker. Studies on this topic on general population have reported an effect on different fecundity related outcomes: NO<sub>2</sub> during the 60 days before the end of the first month of unprotected intercourse was associated with reduced fecundability in Czech Republic (fecundability ratio per each increase by 10 µg/m<sup>3</sup> 0.71, 95% CI 0.57;0.87, Slama et al., 2013), long term exposure to PM was not clearly associated with incident infertility in the US (Mahalingaiah et al., 2016) and PM<sub>coarse</sub> was associated with reduce fertility rates in Spain (infertility risk estimate per interquartile range in yearly PM<sub>coarse</sub>: 0.88, 95% CI 0.83;0.94, (Nieuwenhuijsen et al., 2014). In animals, a study in Sao Paulo observed longer timer to mating in mice living in an exposure chamber exposed to non-filtered PM and decreased fertility (number of cohabited females becoming pregnant divided by total number of mated females) and pregnancy (number of females delivering live pups divided by number of females with evidence of pregnancy) indexes (Veras et al., 2009). At the exposure levels observed in France at the beginning of the 21<sup>st</sup> century, we observed no association but the levels of air pollution were low and from different sources than those of the Teplice area studied by Slama et al. (2013). Identically, the mice in non-filtered chambers in Sao Paulo were living in a traffic area (approximately 100,000 vehicles per days) at levels that might have been reached by only few of our study participants. The fact that the current duration and the prevalent cohort analyses have coherent results in term of direction of the associations and in term of confidence intervals gave confidence for the fact that a not strong association is observed at levels encountered in France, but, as the current duration design results might have been prone to several biases and as the confidence intervals for the prevalent cohort results are wide, it is not clear if such an association really exists and it would be interesting to see if same conclusion on fecundity would appear from studies in other countries.

### 3. Are the reported effects of air pollution on preterm delivery a statistical artefact?

As we saw above with the example of the studies reviewed by Sun et al. (2015), the statistical methods used to study the association between air pollution and preterm birth risk are most of the time inappropriate for exposure windows such as the whole pregnancy and the third trimester of pregnancy. Yet, the two studies (out of 18 included in the review) with adequate statistical methods highlighted an association between whole pregnancy (Chang et al., 2015; Huynh et al., 2006) or third trimester (Chang et al., 2015) exposure to PM<sub>2.5</sub>. Additionally, a recent study conducted in New York with the statistical method than used by Chang et al. (2015) was published on the association of NO<sub>2</sub> and PM<sub>2.5</sub> and preterm birth risk (Johnson et al., 2016). No association was observed in this study with both pollutants nor during first and second trimesters of pregnancy using a logistic regression, neither during third trimester with the survival model (whole pregnancy exposure has not been studied), in line with our results. The OR of preterm birth reported with PM<sub>2.5</sub> exposure during third trimester (T3) and whole pregnancy (WP) in Chang et al, Huynh et al, Johnson et al and our study are summarized in Figure VI-1.

**Figure VI-1: Exposure to PM<sub>2.5</sub> during whole pregnancy and preterm delivery in studies using a survival model or a matched case-control.**



In the Atlanta study (Chang et al., 2015), fixed-cohort bias occurring when study population is included by birth date (Strand et al., 2011), similar to the bias occurring in pregnancy-based approach to study fecundity in the presence of shorter/longer time to pregnancy at the beginning/end of the study period) was avoided, some covariates were available (including maternal smoking, but not maternal height and weight) and the analyses were adjusted on weekly temperature. However, temperature was not studied at other exposure windows than the one considered for atmospheric pollution. In the Californian study (Huynh et al., 2006), each case was matched to three full-term controls with a last menstrual period within 2 weeks of the case, which can have limited the exposure contrast due to temporal variations of air pollution. Smoking was not considered -it was only available for women with pregnancy complications- nor was temperature. Both studies used nearest monitor to define exposure at birth address only, and no information was available on onset of labour or spontaneous versus C-section delivery. In the New York study (Johnson et al., 2016), only non-smoking mother were considered, fixed-cohort bias was avoided, preterm births were categorized in spontaneous versus medically induced, temperature was considered and exposures were derived from temporally adjusted LUR models.

As the percentage of preterm delivery in the US is high (11% in Atlanta, 12% in the Consortium on Safe Labor (Ha et al., 2016) compared to Europe (5% in the ESCAPE study, below 7% for all cohorts except the Greek RHEA cohort with 12% of preterm deliveries, which are born at 67% by C-section), it might be possible that the cause for preterm birth in Europe and USA is different and that the association found in those two US study is not existing in European cohorts. Whenever possible categorizing preterm birth either as spontaneous/medically induced would be important in future studies. Interestingly, the rate of preterm birth in New York City study was not too high compared to Europe (7%) and the conclusion regarding atmospheric pollution was the same than us using a similar exposure model (Johnson et al., 2016). Although this study and ours add weight in the direction of no association, the difference of results with studies from Chang et al. and Huynh et al. highlighted that this association might exists. A possibility exists that even if pregnant women spend in general more time at home than other people, the bias towards null association due to not considering daily mobility have impacted more our results and those of Johnson *and colleagues* (2016) than the results of Chang *and colleagues* and Huynh *and colleagues* as Setton *and colleagues* (2011) observed that in a population not constituted of pregnant women, more bias is observed with an higher resolution exposure model (Setton et al., 2011).

## V. Conclusion

This work based on OBSEFF and ESCAPE studies lends support for an adverse effect of air pollutants on menstrual cycle; it does not bring firm evidence in one direction or the other regarding the currently limited evidence in favor of a deleterious effect of atmospheric pollution on couple's fecundity. It brings direct and indirect (through identification of a specific bias related to exposure windows definition in the published studies) evidence that air pollution does not increase preterm delivery risk, at least in the European setting, contrarily to the common perception of the existing literature. As many of the previous studies, we were not able to consider time space activity in our exposure metric and assessed exposures retrospectively. We showed that using exposure windows with different durations between cases and non-cases is a source of bias in preterm birth studies. To avoid this bias, future studies on the association between preterm birth and air pollutants and meteorological parameters should use a survival model with time-varying exposures, and also, given the possible effects of temperature and atmospheric pressure on preterm birth risk, futures studies of atmospheric pollution effects should consider both atmospheric pollutants and meteorology simultaneously. Studies on fecundity and menstrual cycle need to be replicated in other populations.







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# Annexes



## I. Publications and communications

## 1. Publications and communications related to the thesis

## a. Accepted article

***The Influence of Atmospheric Pollutants and Meteorological Factors on the Risk of Preterm and Very Preterm Birth in Humans.***

Giorgis-Allemand L, Pedersen M, Bernard C, Aguilera I, Ballester F, Beelen RMJ, Chatzi L, Cirach M, Danileviciute A, Dedele A, van Eijsden M, Estarlich M, Fernández-Somoano A, Fernández MF, Forastiere F, Gehring U, Grazuleviciene R, Gruziova O, Heude B, Hoek G, de Hoogh K, van den Hooven EH, Håberg SE, Jaddoe V, Korek M, Lertxundi A, Lepeule J, Nafstad P, Nystad W, Patelarou V, Porta D, Postma D, Raaschou-Nielsen O, Rudnai P, Sunyer J, Stephanou E, Sørensen M, Tuffnell D, Varró MJ, Vrijkotte TJ, Wijga A, Wright J, Nieuwenhuijsen MJ, Pershagen G, Brunekreef B, Kogevinas M, Slama R, In press, Am J Epidemiol.

***Environmental Factors and Preterm Birth: Paths Forwards***

Giorgis-Allemand L, Pedersen M, Beelen RMJ, Gehring U, Hoek G, Basagana X, Nieuwenhuijsen M, Brunekreef B, Kogevinas M, Slama R. In press, Am J Epidemiol.

## b. Articles in preparation

***Atmospheric pollution and characteristics of the menstrual cycle.***

Giorgis-Allemand L, Thalabard JC, Rosetta L, Malherbe L, Meleux F, Siroux V, Bouyer J, Slama R, in preparation

***Does exposure to air pollutants have a short-term impact on time to pregnancy? A study in the general population.***

Giorgis-Allemand L, Pittion F, Bottagisi S, Sorensen D N, Bohet A, Ducot B, Keiding N, Bouyer J, Slama R, in preparation

## c. Oral communications

***Short-term association between atmospheric pollution and the menstrual cycle.***

Giorgis-Allemand L, Bouyer J, Rosetta L, Thalabard J-C, Slama R. 28<sup>th</sup> conference of the International Society for Environmental Epidemiology, 2016, Roma, Italy.

***Do Air Pollutants Have a Short-Term Influence on Couples' Fecundity? An Analysis Relying on Three Novel Study Designs in Parallel.***

Giorgis-Allemand L, Pittion F, Bottagisi S, Sorensen D N, Bohet A, Ducot B, Keiding N, Bouyer J, Slama R, 26<sup>th</sup> conference of the International Society for Environmental Epidemiology, 2014, Seattle, USA.

***Impact à court terme de la pollution atmosphérique sur la fertilité des couples***

Giorgis-Allemand L, Bouyer J, Keiding N, Slama R. Colloque "Bilan et perspectives" des projets finances en 2007 par l'ANSES dans le cadre de l'appel à projets Environnement-Santé-Travail, 2012, Paris, France

d. *Poster communications****Do air pollutants have a short-term influence on time to pregnancy? A current-duration analysis in the general population.***

Giorgis-Allemand L, Pittion F, Bottagisi S, Sørensen DN, Bohet A, Ducot B, Keiding N, Bouyer J, Slama R. 24<sup>th</sup> conference of the International Society for Environmental Epidemiology, 2012.

## 2. Other publications

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II. Comment from Ha and Mendola (American Journal of Epidemiology)

**Invited Commentary: Ambient Environment and the Risk of Preterm Birth**

Authors: Sandie Ha and Pauline Mendola

Available online at <https://academic.oup.com/aje/article-lookup/doi/10.1093/aje/kww138>.

III. Reply to Ha and Mendola.

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**Environmental Factors and Preterm Birth: Paths Forwards**

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*Abbreviations*

CI: Confidence Interval

ESCAPE: European Study of Cohorts for Air Pollution Effects

LUR: Land-Use Regression

OR: Odds-Ratio

*Running head:*

Environmental Influences on Preterm Birth

*Abstract*

The identification of environmental influences on preterm birth risk is a conundrum. We discuss possible paths forwards, taking the example of air pollution as the exposure of interest. The spatial resolution of exposure models has been improved, with many recent studies relying on Land-Use Regression or dispersion models; further refinement of the spatial resolution of models is unlikely to bring more robust results, lest subjects' time space activity is incorporated. The outcome definition generally considers preterm birth as a homogeneous outcome, while it may be more relevant to consider separately preterm births with different underlying maternal-fetal conditions as distinct (competing) outcomes, in a survival modeling setting. Survival models furthermore allow avoiding bias occurring in logistic regression models focused on exposure windows until birth, which may lead to averaging exposure over a shorter duration for preterm, compared to term births. This bias is most likely for the third trimester and whole pregnancy exposure windows, which are those for which associations with particulate matter have been most frequently reported. Therefore, it would be important for authors of past studies to repeat their analyses related to these exposure windows using an approach allowing to avoid this potential bias to happen.

We thank Doctors Ha and Mendola for their thoughtful comments (1) on our study of associations of meteorological conditions and air pollution levels on preterm birth risk (2).

Over the last decade, the spatial resolution of atmospheric pollution exposure models has improved. Dr. Ha and Mendola mention, among other models, dispersion models as possibly more relevant alternatives to the Land-Use Regression (LUR) approach used in ESCAPE project in which our study is embedded. It should be noted that, although not explicitly considered by LUR models, meteorological conditions are still indirectly taken into account, through their influence on the local air pollution levels, which constitute an entry parameter of our seasonalized LUR model (2, 3). A detailed comparison of the yearly estimates of LUR and dispersion models estimates at the home address done in ESCAPE project showed a median (Pearson R) correlation of 0.75 for nitrogen dioxide, and 0.29 for fine particulate matter, with stronger agreement in areas where dispersion models were more predictive of the local measurements done to define LUR models (4). These models should not be opposed, and estimates from dispersion and other models can actually be fed into LUR models to increase their predictive ability (5).

Further improving the spatial resolution of models below the 10-100 m value of typical current models is unlikely to significantly improve accuracy in exposure estimates as long as the time-space activity of pregnant women is not considered (11). In a small scale study of pregnant women carrying GPS devices in a mid-size French city, incorporating time-space activity in an exposure estimate based on an (outdoor) dispersion model entailed little change in exposure estimates for fine particulate matter (a pollutant with limited spatial variability within urban areas), and somewhat larger but still limited changes for NO<sub>2</sub> exposure estimates (12). More generally, it has been shown that bias in dose-response functions due to ignoring time-space activity is likely to increase as spatial resolution of exposure models becomes finer (11).

Taking into account indoor air pollution levels seemed to have a greater impact on exposure estimates (12), which is coherent with the limited correlation reported between personal and outdoor exposures outside the context of pregnancy (13). However, dosimeters cannot easily be carried more than a few weeks during the pregnancy, thus offering a better consideration of indoor levels at the cost of a decreased ability to test numerous exposure windows during pregnancy. Modeling indoor infiltration of outdoor pollutants could be a way to better take indoor levels into account without decreasing the temporal resolution of exposure estimates (14).

Considering larger areas is an option to increase the sample size. As rightly pointed out by Ha and Mendola, there is no consensus as to the best way to correct for bias possibly resulting from the consideration of large study areas (15). The main concern here relates to confounding bias. In the context of a birth cohort, Pedersen et al showed that, as the area considered around the city centers was extended, thus increasing sample size, so did the heterogeneity of the population in terms of disease risk factors, thus increasing the potential for bias in a situation where all confounders cannot be perfectly measured. This is but an illustration of the well-known bias-variance tradeoff (15). We chose to adjust for the study area using a random effect covariate (2). In the case of associations with first trimester atmospheric pressure, not adjusting for center at all did not yield to an increased point estimate (odds-ratio, OR, for an increase by 5 mBar, 1.04, compared to 1.06 (2) after control for center with a random effect variable); taking center into account might indeed induce over adjustment, but allows reducing confounding bias due to preterm birth risk factors varying between areas. Alternatives exist (16) and further work is needed to identify the least biased approach to take center and, more generally unmeasured spatially-varying confounders into account.

We considered preterm (before 37 gestational weeks) and very preterm births (before 32 completed gestational weeks). As suggested by Ha and Mendola, we now considered early term birth (those occurring at 37-38 weeks, taking births from 39 weeks onwards as the reference group) risk. These analyses indicated a monotonous association between early term birth risk and temperature averaged until week 37 (OR, 1.24 for each increase by 10°C, 95% confidence interval, CI, 1.06, 1.46), no association with pressure first trimester average (p, 0.53), i.e. a different pattern than that observed for preterm birth risk (2). Discerning preterm birth cases according to the underlying maternal, placental or fetal conditions would be another relevant step in the future.

Our study provided an illustration of bias that can arise when analyzing associations of preterm birth risk with exposures during a time window spanning until birth, as is the case of exposures during the third trimester of pregnancy. When associations with this exposure window were estimated using a statistical model that did not accommodate time-varying exposures, such as logistic regression, a clear bias was highlighted for temperature, compared to a survival analysis (2). Such a bias may occur for other time-varying exposures such as

exposure to atmospheric pollutants. A meta-analysis indicated that there was no overall evidence for variations in preterm birth risk with particulate matter levels assessed either in the first or second trimesters of pregnancy (17). The meta-analysis was in support of a detrimental association only for the third trimester and whole pregnancy exposure windows (17). These are precisely the exposure windows for which the potential for the bias related to exposures of term and preterm births being averaged over different durations exist when logistic regression is used, which seems to have been the case for most of these studies. For these reasons, we believe an important step to move the question forward would be for authors of past studies to repeat their analyses of last trimester and whole pregnancy exposure windows using a survival approach or any other approach allowing to avoid the potential for this bias to happen. This is likely to shed some light on the important but still dusky area of air pollution effects on preterm birth risk.

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# Résumé

Une fraction importante de la population est exposée à la pollution atmosphérique ; ses effets sur la mortalité et la morbidité cardiovasculaire et respiratoire sont connus, et un effet de l'exposition au cours de la grossesse sur le poids de naissance et la croissance fœtale est probable ; un effet sur le risque de naissance prématurée a aussi été suggéré par de nombreuses études, essentiellement en Amérique. En revanche, la capacité des couples à concevoir -fertilité- et les paramètres de la fertilité féminine ont été très peu étudiés en lien avec cette exposition.

L'objectif de ce doctorat était de documenter un effet éventuel de la pollution atmosphérique sur la fonction de reproduction humaine et tout particulièrement sur les caractéristiques du cycle menstruel, la probabilité de survenue d'une grossesse (fertilité) et le risque de naissance prématurée. Nous nous sommes appuyés sur une cohorte de couples n'utilisant pas de méthode contraceptive (l'Observatoire de la fertilité en France) et sur treize cohortes de naissances européennes participant au projet ESCAPE (European Study of Cohorts for Air Pollution Effects).

Nous avons observé un allongement de la durée de la phase folliculaire du cycle menstruel (période du cycle entre le début des règles et l'ovulation) avec l'exposition de la femme aux particules en suspension dans l'atmosphère ( $n=158$ ,  $\beta=1,6$  jour pour une augmentation de la concentration des particules de diamètre aérodynamique inférieur à  $10 \mu\text{m}$  - $\text{PM}_{10}$ - de  $10 \mu\text{g}/\text{m}^3$  dans le mois précédant le cycle, intervalle de confiance, IC à 95%, 0,3; 2,9). En utilisant deux designs d'étude en parallèle sur la même population, l'approche des durées en cours et l'approche de cohorte prévalente, nous avons mis en évidence une tendance à une diminution de la probabilité de grossesse en association avec l'exposition à la pollution atmosphérique pour la première approche (cohorte prévalente :  $n=468$ , risque relatif de grossesse, HR : 0,69 pour une augmentation des  $\text{PM}_{10}$  de  $10 \mu\text{g}/\text{m}^3$  dans les 70 jours précédant l'inclusion, IC à 95%, 0,43; 1,12) ; la tendance était similaire avec l'approche des durées en cours ( $n=516$ , durée médiane sans contraception multipliée par 1,29 pour une augmentation des  $\text{PM}_{10}$  de  $10 \mu\text{g}/\text{m}^3$  dans les 70 jours précédant l'arrêt de la contraception, IC à 95%, 0,97 ;1,70). Le risque de naissance prématurée, analysé avec un modèle de survie en prenant en compte l'exposition comme une variable dépendant du temps, n'était pas associé à divers polluants atmosphériques dans les cohortes du projet ESCAPE ( $n=46\ 791$ , OR=0,97 pour une augmentation du niveau moyen de  $\text{PM}_{10}$  de  $10 \mu\text{g}/\text{m}^3$  pendant la grossesse, IC à 95%, 0,87 ;1,07). Nous avons par ailleurs mis en évidence une augmentation du risque de naissance prématurée avec la pression atmosphérique pendant le premier trimestre de grossesse et avec la température moyenne pendant le premier trimestre, au moins dans l'intervalle entre  $-5^\circ\text{C}$  et  $10^\circ\text{C}$ . Nous avons montré qu'une partie de la littérature en faveur d'une association entre particules fines et risque de naissance prématurée pourrait être sujette à un biais causé par des durées de fenêtres d'exposition différentes entre les enfants nés avant terme et ceux nés à terme.

Dans l'ensemble, ce travail confirme la nécessité d'utiliser un modèle de survie avec variables dépendant du temps pour étudier le risque de naissance prématurité et appelle à poursuivre les recherches concernant des effets possibles des polluants atmosphériques sur le cycle menstruel et la fertilité, pour lesquels nos travaux font partie des premiers réalisés en population générale.

**Mots clés :** *pollution atmosphérique, reproduction humaine, cycle menstruel, fertilité, prématurité*

A large fraction of the population is exposed to atmospheric pollution, which has known effects on cardiovascular and respiratory mortality and morbidity and probable effect on birthweight and fetal growth. So far, the biological aptitude to conceive for couples -fecundity- and the female markers of fecundity have been seldom studied in relation with this environmental exposure.

The aim of this thesis was to quantify the possible association between atmospheric pollution and specific health outcomes related to human reproduction: menstrual cycle characteristics, probability of pregnancy and preterm birth risk. We relied on a population of couples not using any contraceptive method (Observatory of Fecundity in France) and on 13 birth cohorts participating in the European Study of Cohorts for Air Pollution Effects.

We observed that higher levels of atmospheric pollutants during the 30 days before the start of a menstrual cycle were associated with longer follicular phase ( $n=158$ ,  $\beta=1.6$  days per each increase by  $10 \mu\text{g}/\text{m}^3$  in particulate matters with an aerodynamical diameter of less than  $10 \mu\text{m}$  - $\text{PM}_{10}$ ; 95% confidence interval: 0.3;2.9). In the population recruited in OBSEFF study, we observed a trend for an increased time to pregnancy with short-term  $\text{NO}_2$  and  $\text{PM}_{10}$  levels in an original approach relying on two seldom used study designs focusing on a marker of fecundity in parallel: the prevalent cohort approach ( $n=468$ , hazard ratio of pregnancy, HR: 0.69 per each increase by  $10 \mu\text{g}/\text{m}^3$  in  $\text{PM}_{10}$  during the 70 days before the inclusion, with a 95% CI of 0.43;1.12) and the current duration approach ( $n=516$ , median current duration of unprotected intercourse multiplied by 1.29 per each increase by  $10 \mu\text{g}/\text{m}^3$  in  $\text{PM}_{10}$  during the 70 days before the contraception stop, 95% CI: 0.97;1.70). In the cohorts included in ESCAPE, preterm delivery risk studied by a survival model with time-dependent exposures was not associated with atmospheric pollutants levels during pregnancy ( $n=46,791$ , OR=0.97 per each increase by  $10 \mu\text{g}/\text{m}^3$  in  $\text{PM}_{10}$  during the whole pregnancy, 95% CI 0.87;1.7). We observed an increased risk of preterm birth with higher atmospheric pressure during the first trimester of pregnancy and to some extent with temperature between  $-5^\circ\text{C}$  and  $10^\circ\text{C}$  during the first trimester of pregnancy. We additionally showed that using exposure windows with different durations between cases and non-cases is a source of a bias in preterm birth studies that may impact several studies in the literature.

This work demonstrated that using a survival model with time-dependent exposures is crucial to study preterm delivery risk. It appeals for additional research on the possible adverse effects of atmospheric pollution on menstrual cycle and fecundity, as our studies are among the first ones conducted in a general population on those topics.

**Key words :** *atmospheric pollution, human reproduction, menstrual cycle, fecundity, preterm delivery*