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Population-based registries to assess environmental health risks and to evaluate public health measures

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Summary

Nearly a quarter of the global disease burden and premature mortality is expected to be caused by environmental exposures, and the environmental health burden in children is even larger. Among the most hazardous environmental risk factors are tobacco smoke and ambient air pollution. Also temperature extremes have been linked to different adverse health outcomes, which is a concern because of the expected increase in extreme weather events due to climate change.

It is well accepted that prenatal and postnatal exposure to second-hand tobacco smoke has detrimental effects on fetal and child health. However, evidence that smoke-free legislations are associated with health benefits early in life was limited before 2013. Also early life health effects of ambient temperature and air pollution are a relatively novel research domain. The number of studies investigating the association between temperature and preterm birth is limited, and results are inconclusive. Concerning fetal growth, evidence for adverse effects of air pollution is cumulating, but results are inconsistent with respect to the exposure window. Finally, although animals may serve as useful sentinels for the effects of environmental exposures on human health, epidemiological findings for human have seldom been replicated in animal populations.

In this dissertation, we used population-level registry data to investigate health effects of different environmental exposures and to evaluate the health impact of preventive public health measures, including health outcomes in newborns and in the older segment of the population. The specific objectives of this dissertation were:

1. The assessment of potential public health benefits of smoking policies, focusing on health outcomes in early and in later life. For this purpose, we studied the impact of the stepwise implementation of smoke-free legislation in Belgium on the risk of preterm birth and on the risk of mortality due to acute myocardial infarction.
2. The investigation of the association between environmental exposures and adverse birth outcomes. First, we examined the risk of preterm birth in relation to ambient temperature by investigating potential heat and cold effects. Second, we studied fetal growth in relation to maternal exposure to PM_{10}. For the latter, we focused on birth weight and small-for-gestational-age and we

assessed potential effect modification by pregnancy duration and potential non-linearity in the exposure-response shape.

3. The exploration of the use of dairy cattle populations as comparative models of human health risks. For this purpose, we investigated the association between dairy cow mortality and ambient O_3, PM_{10}, NO_2 and temperature, accounting for harvesting and delayed effects.

Using data on all live born singletons in Flanders between 2002 and 2011, we found that the stepwise implementation of smoke-free legislation was associated with subsequent reductions in the risk of preterm birth (**chapter 2**). We observed an immediate (step) change in the risk of spontaneous preterm delivery on 1 January 2007 (ban on smoking in restaurants), and a gradual (slope) change after 1 January 2010 (ban on smoking in bars serving food). The analysis for all births gave similar results. This finding supports the notion that smoking bans have public health benefits from early life onwards.

In **chapter 3**, we showed that the smoking policy in Belgium also has beneficial health impacts in an older segment of the population. Based on data from all acute myocardial infarction (AMI) deaths of 30 years or older in Flanders between 2000 and 2009, we observed an immediate decrease in AMI mortality rates in January 2006 (smoking ban at work). The effect was highest for women younger than 60 years. An additional effect of the smoking ban in restaurants was observed for men aged 60 years or older, showing a slope change in AMI death rates since January 2007.

Findings of our study on the association between temperature and preterm birth in the temperate climate of Flanders are presented in **chapter 4**. Using data on singleton live-born natural births between 1998 and 2011, we found an increased risk of preterm birth in the warm season when minimum temperature up to 3 days before delivery (lag 0–3) exceeded the 95th percentile. In the cold season, we observed an increase in the risk of preterm birth associated with low minimum temperature two days before birth (lag 2), but cumulative cold effects were not significant.

Chapter 5 focuses on maternal exposure to particulate matter (PM_{10}) and its effect on fetal growth among singleton live births in Flanders between 1999 and 2009. Among babies born after 31 weeks of gestation, we found a consistent

association between birth weight and maternal PM_{10} exposure during all studied time windows. Also the risk of being small-for-gestational-age was significantly associated with maternal PM_{10} exposure during most of the time windows. Effects tended to be stronger for moderately preterm births (32-36 weeks) than for term births (>36 weeks). Segmented regression models showed stronger effects of PM_{10} on fetal growth at lower concentrations.

In **chapters 6 and 7**, we investigated day-to-day variations in dairy cattle mortality in association with day-to-day variations in air pollution and temperature respectively. We used data on dairy cow deaths in Belgium from 2006 to 2009 and we accounted for potential harvesting and delayed effects up to 25 days after exposure. We found significant effects of air pollution and temperature on dairy cow mortality during the warm half of the year, but not during the cold period. Acute air pollution effects (up to lag 0–1) were significant for O_3 and NO_2 and were largest for NO_2. For O_3 and PM_{10} we observed additional delayed effects and (significant) cumulative 26-day (lag 0 to 25) estimates were considerably larger than the acute effects. Heat effects were acute and were followed by a deficit in mortality 3 to 5 days after the exposure. Taking into account this harvesting effect, there was still a net increase in mortality. We also observed a significantly increased mortality risk associated with low temperatures in the warm season. Cold effects were delayed by 5 days and persisted up to 18 days after the exposure.

The advantages and disadvantages of the use of secondary registry data are discussed in **chapter 8**, in addition to a discussion of our findings in relation to the available literature, and the public health implications of our work.

Samenvatting

Men schat dat bijna een kwart van de globale ziektelast en vroegtijdige sterfte veroorzaakt wordt door blootstelling aan omgevingsfactoren, en de impact van het milieu op de gezondheid van kinderen is zelfs nog groter. Tabaksrook en luchtverontreiniging behoren tot de meest schadelijke omgevingsblootstellingen. Ook blootstelling aan zeer lage of hoge buitentemperaturen kan leiden tot verscheidene nadelige gezondheidseffecten, wat een probleem vormt in de context van de verwachte toename in extreme weersomstandigheden door de klimaatsverandering.

Het is algemeen aanvaard dat prenatale en postnatale blootstelling aan tabaksrook schadelijk zijn voor de gezondheid van de foetus en het kind. Toch was er vóór 2013 nog bijna geen wetenschappelijk bewijs dat rookvrije wetgevingen al vroeg in het leven gezondheidswinst opleveren. Ook het onderzoek naar perinatale gezondheidseffecten van omgevingstemperatuur en luchtverontreiniging is relatief nieuw. Slechts een beperkt aantal studies onderzocht het verband tussen temperatuur en vroeggeboortes, en de resultaten zijn niet eenduidig. Hoewel er groeiend wetenschappelijk bewijs is dat blootstelling aan luchtverontreiniging schadelijk is voor de groei van de foetus, zijn de resultaten van studies inconsistent wat betreft de periode van de zwangerschap waarin de foetus het meest gevoelig is aan zulke blootstelling. Hoewel dieren kunnen gebruikt worden als rolmodellen voor de effecten van milieufactoren op de gezondheid van de mens, werden epidemiologische bevindingen bij de mens slechts zelden bevestigd in dierenpopulaties.

In dit proefschrift gebruikten we registergegevens op populatieniveau om de gezondheidseffecten van verscheidende milieufactoren te onderzoeken en om de impact van preventieve volksgezondheidmaatregelen te evalueren. Hiervoor keken we naar gezondheidsuitkomsten in pasgeborenen en in een ouder deel van de populatie. De specifieke doelstellingen van dit proefschrift waren:

1. Een inschatting maken van mogelijke gezondheidsvoordelen ten gevolge van rookwetgevingen, gericht op aandoeningen zowel vroeg als later in het leven. Hiervoor onderzochten we de impact van de stapsgewijze invoering van het rookverbod in België op het risico op vroeggeboorte en op het risico op sterfte door een hartaanval.

2. Het onderzoeken van het verband tussen omgevingsblootstellingen en negatieve zwangerschapsuitkomsten. Om te beginnen bestudeerden we de effecten van lage en hoge buitentemperaturen op het risico op vroeggeboorte. Daarnaast gingen we na of er een verband is tussen foetale groei en blootstelling van de moeder aan luchtverontreiniging. Hiervoor keken we naar geboortegewicht en naar het risico om te licht te zijn rekening houdend met de zwangerschapsduur ("small-for-gestational-age"). We keken ook naar mogelijke effect modificatie door zwangerschapsduur en mogelijke niet-lineariteit in het verband tussen blootstelling en uitkomst.
3. De exploratie van het gebruik van melkkoeien als vergelijkende modellen voor menselijke gezondheidsrisico's. Hiervoor onderzochten we de associatie tussen sterfte bij melkkoeien en concentraties van ozon (O_3), fijn stof (PM_{10}) en stikstofdioxide (NO_2) in de buitenlucht. Daarnaast keken we ook naar de effecten van extreme buitentemperaturen. In de analyses hielden we rekening met mogelijke "harvesting" en vertraagde blootstellingseffecten.

Gebruik makend van gegevens van alle levend geboren eenlingen in Vlaanderen van 2002 tot 2011 vonden we dat de stapsgewijze invoering van het rookverbod geassocieerd was met opeenvolgende reducties in het risico op vroeggeboorte (**hoofdstuk 2**). We zagen een onmiddellijke daling in het risico op spontane vroeggeboorte op 1 januari 2007 (rookverbod in restaurants), en een graduele verandering vanaf 1 januari 2010 (rookverbod in brasserieën). De inclusie van geïnduceerde bevallingen in de analyse gaf gelijkaardige resultaten. Deze bevindingen ondersteunen het vermoeden dat rookvrije wetgevingen al vroeg in het leven een gezondheidswinst opleveren.

In **hoofdstuk 3** toonden we dat het rookverbod in België ook een gunstig effect heeft op de gezondheid van volwassenen. Op basis van gegevens van alle sterfgevallen door een acuut myocard infarct (AMI) bij mensen van 30 jaar of ouder in Vlaanderen van 2000 tot 2009 zagen we een onmiddellijke daling in AMI sterfte in januari 2006 (rookverbod op de werkvloer). Deze daling was het sterkst bij vrouwen jonger dan 60 jaar. Bij mannen van 60 jaar of ouder vonden we een bijkomend effect van het rookverbod in restaurants, namelijk een geleidelijke afname in sterfte door AMI sinds januari 2007.

De resultaten van onze studie over het verband tussen temperatuur en vroeggeboorte in het gematigde klimaat van Vlaanderen zijn terug te vinden in **hoofdstuk 4**. Gebruik makend van gegevens van natuurlijke bevallingen van levend geboren eenlingen van 1998 tot 2011 vonden we een verhoogd risico op vroeggeboorte in het warme seizoen wanneer de minimum temperatuur tot 3 dagen voor de bevalling (lag 0–3) het 95ste percentiel overschreed. In het koude seizoen zagen we een toename in het risico op vroeggeboorte geassocieerd met een lage minimum temperatuur 2 dagen voor de geboorte (lag 2), maar cumulatieve koude-effecten waren niet significant.

Hoofdstuk 5 gaat over maternale blootstelling aan fijn stof (PM_{10}) en het effect hiervan op foetale groei bij levend geboren eenlingen in Vlaanderen van 1999 tot 2009. Bij baby's waarvan de zwangerschapsduur meer dan 31 weken bedroeg vonden we een consistente associatie tussen geboortegewicht en blootstelling aan fijn stof tijdens alle bestudeerde tijdsvensters van de zwangerschap. Ook het risico om te licht geboren te worden voor de zwangerschapsduur toonde een significant verband met blootstelling aan fijn stof voor het merendeel van de onderzochte zwangerschapsperiodes. De effecten leken sterker te zijn voor matig premature geboortes (32-36 weken) dan voor voldragen geboortes (>36 weeks). Breekpunt ("segmented") regressie modellen toonden dat de effecten van PM_{10} op foetale groei sterker waren bij lagere dan bij hogere concentraties.

In **hoofdstukken 6 en 7** onderzochten we dagelijkse variaties in sterfte bij melkkoeien in relatie tot respectievelijk dagelijkse variaties in luchtverontreiniging en temperatuur. We gebruikten gegevens van sterfte bij melkkoeien van 2006 tot 2009 en we hielden rekening met mogelijke harvesting en vertraagde effecten tot 25 dagen na de blootstelling. We vonden significante stijgingen in sterfte bij melkkoeien geassocieerd met luchtverontreiniging en temperatuur tijdens de warme periode van het jaar, maar niet tijdens de koude periode. Acute effecten van luchtverontreiniging (tot lag 0–1) waren significant voor O_3 en NO_2 en waren het grootst voor NO_2. Voor O_3 en PM_{10} zagen we bijkomende vertraagde effecten en de (significante) cumulatieve schattingen over 26 dagen (lag 0 tot 25) waren aanzienlijk groter dan de acute effecten. Hitte-effecten waren acuut en werden gevolgd door een ondersterfte 3 tot 5 dagen na de blootstelling. Dit harvesting effect in rekening nemend was er nog steeds een netto stijging in sterfte. We zagen ook een significante stijging in sterfte geassocieerd met lage temperaturen

in het warme seizoen. Deze koude-effecten traden pas op na 5 dagen en duurden voort tot 18 dagen na de blootstelling.

De voor- en nadelen van het gebruik van gegevens van secundaire registers worden besproken in **hoofdstuk 8**, naast een discussie van onze bevindingen in relatie tot de beschikbare literatuur, en de relevantie ervan voor de volksgezondheid.

Contents

Chapter 1

General introduction

In the top ten of leading diseases in terms of global disease burden in 2010 are conditions such as ischemic heart disease, lower respiratory tract infections, preterm birth complications and chronic obstructive pulmonary disease (COPD).[1] These diseases are also in the top ten of diseases with the largest contribution of the environment.[2] According to estimates of the World Health Organization (WHO) as much as 24% of the global disease burden (healthy life years lost) and 23% of all deaths (premature mortality) was caused by environmental exposures.[3] Children suffer a disproportionate share of the environmental health burden: up to the age of 14 years, the proportion of deaths attributed to the environment was as high as 36%.[3]

The actual contribution of the environment to non-communicable diseases might even be underestimated because of two reasons.[4] Firstly, lifestyle behavioral risk factors have been excluded as environmental risk factors because they are considered to involve an element of choice and individual responsibility. However, especially for children, exposure to lifestyle risk factors like diet and tobacco smoke are not lifestyle choices but rather environmental exposures imposed on them by others. Secondly, estimates of environmental health burden do not capture the contribution of early-life exposures to the development of chronic diseases later in life because these connections are often difficult to establish due to the long lag period between exposure and outcome. A better understanding of environment-health interactions is important because well-targeted interventions can prevent much of the environmental risk.[2]

Tobacco smoke, including exposure to second-hand smoke (SHS), was ranked as the second leading risk factor in terms of global disease burden in 2010.[1] Exposure to ambient particulate-matter (PM) pollution was ranked 9th among the risk factors.[1] In the WHO global burden of disease study of 2000, also climate change was in the list of 26 leading environmental risk factors,[5] because of the predicted increase in the frequency and intensity of heat waves, the worsening of air quality, the increase in floods and droughts, changes in the distribution of vector-borne diseases and effects on the risk of disasters and malnutrition.[6] Although climate change has been declared to be the biggest global health threat of the 21st century,[7] this statement has been rejected by others because climate change cannot be seen as a stand-alone risk factor, but rather as an amplifier of existing health and food security risks.[8 9]

Numerous epidemiological studies have demonstrated detrimental effects of exposure to (second-hand) tobacco smoke, air pollution and extreme temperatures on a variety of health outcomes. As many adverse health outcomes are more prominent or only occur at older ages, a lot of studies focused on adults or elderly. More recent evidence, however, suggests that also children and even fetuses suffer from health problems related to environmental tobacco, air pollution and temperature exposure.

1. Tobacco

1.1. Health effects of active and passive smoking

The first prospective evidence on cigarette smoking and lung cancer was published by Doll and Hill in 1954.[10] In 1962, Framingham studies showed that smoking increased the risk of heart disease.[11] Despite the growing evidence indicating tobacco as the major cause of lung cancer and a number of other diseases, the tobacco industries hired scientists to dispute these findings and this battle continued for almost half a century. To date, different meta-analyses have been conducted in which the relative risk estimates from individual studies are pooled. Such meta-analyses have confirmed the association between smoking and different types of cancer, cardiovascular disease, fractures, reduction in fertility, ocular damage, neurological effects, rheumatoid arthritis, and prenatal and postnatal effects in children.[12]

The first conclusive evidence on the danger of passive smoking only came in 1981.[13] Scientific evidence that exposure to SHS leads to premature death and disease is summarized in the 2006 U.S. Surgeon General's report.[14] The WHO estimates that 603,000 deaths were attributable to SHS exposure in 2004, corresponding to 1.0% of the worldwide mortality.[15] Most of the SHS exposure disease burden results from cardiovascular disease, lung cancer, respiratory disease and developmental effects in children.[14]

A large body of evidence indicates that maternal smoking is associated with detrimental effects on child health, such as decreased lung growth and increased rates of respiratory tract infections,[16-18] otitis media,[18 19] asthma,[18 20] sudden infant death syndrome,[18 21] and behavioral and neurocognitive problems.[22 23]

Studies of each of these problems suggest independent effects of both pre- and postnatal exposure.[24] Children are more vulnerable to the adverse effects of environmental tobacco smoke than adults as their lungs and immune system are still developing. The independent effect of prenatal exposure on children's health indicates that maternal smoking in pregnancy influences the fetal development of different organ systems. There is pooled evidence that constituents of cigarette smoke cross the placenta, reduce intra-uterine fetal growth, and increase the risk of preterm delivery and congenital anomalies, not only in smoking mothers,[25-27] but also in non-smoking mothers exposed to SHS.[28-30] Low birth weight and preterm delivery, at their turn, are determinants of health risks later in life, including asthma[31 32] and emotional and behavioral problems.[33]

1.2. Smoke-free legislation

The historical development of smoke-free environments began in the mid-1970s and expanded in the 1990s. Beginning in the early 1980s, results of scientific studies and governmental and intergovernmental reports provided the information needed to promote smoke-free policies. In 2004, Ireland was the first country to implement a comprehensive smoking ban in indoor workplaces, including restaurants and bars. Ten years later (2014), 92 countries have implemented nationwide legislation, although only 62 include bars and restaurants.[34]

A comprehensive review published in 2009 provided consistent evidence that smoking bans were associated with reduced exposure to SHS.[35] A total of 31 studies on self-reported exposure to SHS, mostly in workplaces, showed that smoke-free legislation resulted in a reduction of time exposed (71% to 100% reduction) or a reduction in the proportion of those exposed (22% to 85%).[35] Eighteen studies, using biomarkers like salivary cotinine to validate these self-reports, found a 39% to 89% reduction in exposure.

Results from different meta-analyses, mainly on cardiovascular events,[36-38] suggest that the ultimate goal of smoke-free policies, *i.e.* improving public health, has been accomplished. A recent meta-analysis, based on 45 studies of 33 smoke-free laws, showed that comprehensive smoke-free legislation was associated with significantly lower rates of hospital admissions (or deaths) for coronary events

(relative risk 0.848, 95% CI 0.816 to 0.881), other heart disease (0.610, 0.440 to 0.847), cerebrovascular accidents (0.840, 0.753 to 0.936), and respiratory disease (0.760, 0.682 to 0.846).[39] It should be noted that the observed health benefits are probably not solely due to reduced exposure to SHS, but also due to a decrease in active smoking. A review on tobacco control policies and prevalence of smoking found moderate evidence for a decrease in smoking prevalence (based on 20 studies), although a large majority of the studies showed a significant decrease in prevalence after the ban.[40]

Contrary to the large body of evidence for beneficial effects of smoking bans on adult health, studies focusing on child and fetal health were limited before 2013.[41-45] However, children account for more than a quarter of all deaths and more than half of all disability-adjusted life years due to passive smoking.[15] 40% of children worldwide are regularly exposed to second-hand smoke,[15] which is of great concern because they are generally unable to influence their own level of exposure.

2. Ambient air pollution

2.1. History

Whereas historically air pollution was generally a local issue, air pollution problems have shifted from the local to the global scale along with growing populations, industrialization and fossil-fuel based transportation. Recognition of the dangers of ambient air pollution can be traced to several extreme episodes during the last century. One of the earliest such events occurred in the Meuse Valley in Belgium in 1930. In the last 2 days of the episode, more than 60 persons died, which was more than 10 times the normal mortality rate.[46] Perhaps to most severe such event took place in London in 1952, with an estimated 12,000 excess deaths from December 1952 through February 1953.[47]

Although air pollution from combustion of traditional fossil fuel is much lower than 60 years ago, other components have gained prominence.[48] Airborne particles (PM) have changed size distribution and composition, altering their toxicity. Photochemical air pollution is characterized by high ozone (O_3) concentrations during warm and sunny weather, whereas nitrogen oxides (NO_x) are produced by

the ever rising number of motorized vehicles. Despite regulatory control measures that have lowered concentrations, air pollution continues to harm health. During the late 1980s results of epidemiological studies identified PM less than 10 μm diameter (PM_{10}) as a key pollutant metric related to acute (short-term) and chronic (long-term) health effects. More recently, attention has shifted to measurements and health effects of PM less than 2.5 μm diameter ($PM_{2.5}$), because these particles can penetrate deep into the lungs and bloodstream.

2.2. Health effects of ambient air pollution

Exposure to pollutants such as PM, O_3, and NO_2 has been associated with increases in mortality and hospital admissions due to respiratory and cardiovascular disease. These effects have been found in short-term studies, which relate day-to-day variations in air pollution and health, and long-term studies, which have followed cohorts of exposed individuals over time. Substantial evidence for short-term effects of air pollution on hospital admissions and mortality comes from meta-analyses[49-54] and large-scale multi-city studies such as APHEA (Air Pollution and Health: A European Approach),[55 56] NMMAPS (National Morbidity, Mortality and Air Pollution Study),[57-59] APHENA (Air Pollution and Health: A Combined European and North American Approach),[60 61] and PAPA (Public Health and Air Pollution in Asia).[62] People at highest risk tend to be those with pre-existing vascular disease, diabetes mellitus, and the elderly. Perhaps even more importantly, chronic air pollution exposure significantly elevates the long-term risk for premature cardiopulmonary death throughout the entire population.[63 64]

The WHO has estimated that outdoor air pollution was responsible for 3.7 million deaths in 2012 - roughly 1 in 15 deaths globally.[65] About 88% of these deaths occur in low- and middle-income countries, which represent 82% of the world population. To put this in a wider global perspective, the corresponding estimate for indoor air pollution (from cooking over coal, wood and biomass stoves) was 4.3 million deaths, almost all in low and middle income countries.[65] Estimates suggest that 40% of deaths caused by outdoor air pollution are due to ischemic heart disease, another 40% due to stroke, 11% due to COPD, 6% due to lung cancer, and 3% due to acute lower respiratory infections in children.[65]

More recently, exposure to ambient air pollution has also been linked to several other health outcomes such as atherosclerosis,[66] [67] diabetes,[68] [69] neurodevelopment and cognitive function,[70-72] and adverse birth outcomes. There is meta-analytic evidence for an effect of prenatal air pollution exposure on congenital cardiac anomaly risk.[73] Other recent reviews[74-78] and meta-analyses[79] [80] suggest that maternal air pollution exposure during pregnancy is associated with adverse birth outcomes such as low birth weight, small-for-gestational-age (SGA) and preterm birth. However, they all concluded that reported effects are heterogeneous, especially with respect to the exposure period.

3. Ambient temperature

The impact of meteorological factors on health has gained attention since the record-breaking heat wave in Europe in August 2003. Europe experienced its hottest weather in at least 500 years, which resulted in an estimated 70,000 additional deaths.[81] The 3 years needed to obtain this estimate demonstrated the lack of functional surveillance systems. This has initiated efforts to implement heat plans and real-time monitoring of human mortality at national[82-84] and at European level.[85] [86] Monitoring of all-cause deaths provides a major source of timely data on progression and impact of health threats, which are crucial to guide health service response and public health decision-making.

Temperature-related mortality is a growing public health concern because of climate change. Climate change is projected to increase global mean surface temperatures by 2–4.5°C with 76% probability, and over 4.5°C with 14% probability, by 2100[87] and will increase the frequency and intensity of extreme weather events. Numerous epidemiological studies have shown an association between ambient temperature and total or cause-specific morbidity[88] and mortality,[89] with increased health risks observed during heat waves[90] as well as during cold spells.[91] The most vulnerable population groups are persons with pre-existing conditions, elderly and children.[90-92] Heat-related health effects include heatstroke, hyperthermia, dehydration, renal, cardiovascular, respiratory and nervous system diseases.[90] [93]

More recent evidence suggests that ambient temperature might also affect fetal health. Maternal exposure to extreme temperatures has been linked to adverse

birth outcomes such as low birth weight,[94 95] preterm birth,[96 97] stillbirth,[97] and birth defects.[98] A relatively recent review concluded that the evidence for an adverse effect of high temperatures was stronger for birth weight than for preterm birth and that more research is needed to clarify whether the observed associations are causal.[98]

4. Main objectives

In this doctoral dissertation population-based registries were used to assess the impact of important environmental risk factors on different health outcomes, and to evaluate preventive public health measures. Because environmental risk factors can operate over the entire life span, we used a life-course approach by including both studies on newborns and studies on the older segment of the population, thereby focusing on health outcomes for which certain research gaps have recently been identified. We did not limit ourselves to registries from human populations, but verified human epidemiological findings in registries of cattle which might serve as a sentinel for human public health.

The specific objectives of this study were:

1. The assessment of potential public health benefits of smoking policies, focusing on health outcomes in early and in later life. For this purpose, we studied the impact of the stepwise implementation of smoke-free legislation in Belgium on the risk of preterm birth (chapter 2) and on the risk of mortality due to acute myocardial infarction (chapter 3).
2. The investigation of the association between environmental exposures and adverse birth outcomes. First, we examined the risk of preterm birth in relation to ambient temperature by investigating potential heat and cold effects (chapter 4). Second, we studied fetal growth in relation to maternal exposure to PM_{10} (chapter 5). For the latter, we focused on birth weight and small-for-gestational-age and we assessed potential effect modification by pregnancy duration and potential non-linearity in the exposure-response shape.
3. The exploration of the use of dairy cattle populations as comparative models of human health risks. For this purpose, we investigated the association between dairy cow mortality and ambient O_3, PM_{10} and NO_2 (chapter 6) and temperature (chapter 7), accounting for harvesting and delayed effects.

5. Data

5.1 Perinatal data

Flemish birth data were obtained from the independent and regionally funded Study Centre for Perinatal Epidemiology (SPE).[99 100] The representational status of the SPE data is such that the registration intake is recognized as the equivalent of the legal birth certificate by the government of Flanders (Northern part of Belgium).[101] The SPE collects birth data since 1986, aiming at the collection of data necessary for a routine monitoring of perinatal activities, for international comparisons, and for the guidance of interventions when needed. The SPE collects data on births in all maternity units in Flanders and in the University Hospital of Brussels (UZ Brussel). Also most of the home deliveries are registered. The database includes live births and stillbirths with a birth weight of at least 500 grams, or with a gestational age of at least 22 weeks when the birth weight is unknown. When both birth weight and gestational age are unknown, the criterion is a length of 25 cm or more. The obstetric and perinatal form contains 35 items, which are linked to the 19 items in the neonatal form in case of transfer of the child to a neonatology unit. The official and SPE-encoded form is completed by the midwife or obstetrician who supervised the birth and is subsequently returned to the SPE, where data are checked by an error detection program.

5.2 Acute myocardial infarction mortality

Data on deaths due to acute myocardial infarction in Flanders were obtained from the Flemish Agency for Care and Health.[102] When a person dies, the physician has to record the cause(s) of death on the death certificate, which is then further completed by the civil registry service of the municipality of death. The coding and registration of death causes in Belgium is done independently for Flanders (and Brussels) and Wallonia (including the German-speaking community). The responsible institute for Flanders is the Flemish Agency for Care and Health. Since 1998 causes of death are classified according to the WHO codes of the International Classification of Diseases, 10th revision (ICD-10). Data entries are carefully checked for completeness and accuracy concerning medical and demographical information and apparent inconsistencies are verified by looking up the certificate or by contacting the treating physician.[103] Data for Flanders are

limited to inhabitants of the Flemish region that died in the Flemish or Brussels region. Flemish residents dying in the Walloon region or abroad are not included, resulting in an underestimation of total mortality of about 1%.[104]

5.3 Cattle mortality

Data on dairy cattle mortality in Belgium were extracted from Sanitel, a national-level computerized database for the registration and traceability of farm animals, managed by the Federal Agency for the Safety of the Food Chain.[105] The initial goal of Sanitel was to monitor the health state of the Belgian livestock. The system was later extended to the management of food safety with an eye on the illegal use of growth promoters, residues in milk or chemical contamination. Farmers are legally obliged to register each change in livestock (*i.e.* arrival, departure, birth or death of an animal on their farm) by means of a (self-kept) paper form or an electronic application (Veeportaal or Cerise). Newborn calves have to be earmarked and registered within 7 days, and arrivals and departures (including death) have to be registered within 3 days. In addition, farmers have to report these events to Sanitel within 7 days by sending the passport (in case of death) or notification form, by a phone call (application VRS) or by a notification through the electronic application.

6. Distributed lag non-linear models

In chapters 4, 6 and 7 we used a relatively new statistical method to study the triggering effect of environmental exposures on health outcomes, *i.e.* distributed lag non-linear models (DLNMs). DLNMs enable the investigation of the temporal pattern of the association and provide an estimate of the "overall" effect of the exposure incorporating potential delayed and harvesting effects. Harvesting (or mortality displacement when studying deaths) refers to the situation where adverse health events are only brought forward by a few days in frail individuals who would have suffered the event in the near future anyway.

Numerous studies on the short-term health effects of heat or air pollution have modelled single-day exposures or multi-day moving averages. Many of them have tested different lags or moving averages in separate models, thereby increasing

the type I error, and often the single most significant lag or moving average was selected as final result. Another problem with the use of single lags is that - to the extent that exposure variables are auto-correlated over time - the effects of adjacent lag terms will also be picked up. Many studies found that multi-day moving averages fit better than any single day's exposure, suggesting that the effect of an increase in exposure on a single day is distributed across several subsequent days.

One possible solution to investigate the overall effect of an exposure over many days is to construct an unrestricted (unconstrained) distributed lag model that contains many lagged terms of the exposure as separate variables. However, because of collinearity between the lagged regressors, such model will produce unstable estimates for the individual lags so that no conclusions can be drawn on the shape of the distributed effect.[106 107] Nevertheless, the sum of the individual lag effects will be an unbiased estimate of the overall effect.[106 107] To gain more efficiency and to reduce the noise of the unconstrained model with minimal bias, the coefficients of the lags can be constrained by using a flexible function such as a low degree polynomial.[106] Whereas distributed lag models (DLMs) have been extensively used in the field of social sciences, their first application in epidemiological research dates from 2000 when Schwartz investigated the distributed lag between PM_{10} and daily deaths.[106] To obtain more flexibility in the lag structure, especially when examining longer lags, later studies have used spline functions instead of polynomials and have applied this approach to capture potential harvesting in the estimation of mortality related to air pollution or temperature exposure.[107 108]

Conventional DLMs rely on the assumption of a linear effect between the exposure and the outcome, which is especially problematic for an exposure such as temperature, which typically shows a J- or U-shaped association with health outcomes. Some attempts to relax this assumption have been proposed, such as a constrained segmented parameterization assuming distributed lag linear effects of high and low temperatures beyond the heat and cold threshold respectively.[109] A useful generalization is achieved through the generation of a new model framework which can describe non-linear relationships both in the space of the predictor and along lags, leading to the family of distributed lag non-linear models

(DLNMs).[110 111] This family of models is implemented in the package "dlnm" within the statistical environment R.[112]

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Chapter 2

Impact of a stepwise introduction of smoke-free legislation on the rate of preterm births: analysis of routinely collected birth data

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Abstract

Objective To investigate the incidence of preterm delivery in the Belgian population after implementation of smoke-free legislation in three phases (in public places and most workplaces January 2006, in restaurants January 2007, and in bars serving food January 2010).

Design Logistic regression analyses on routinely collected birth data from January 2002 to December 2011.

Setting Flanders, Belgium.

Population All live born singleton births delivered at 24–44 weeks of gestation (n=606,877, with n=448,520 spontaneous deliveries).

Main outcome measures Preterm birth (gestational age <37 weeks).

Results We found reductions in the risk of preterm birth after the introduction of each phase of the smoking ban. No decreasing trend was evident in the years or months before the bans. We observed a step change in the risk of spontaneous preterm delivery of -3.13% (95% CI -4.37 to -1.87; P<0.01) on 1 January 2007 (ban on smoking in restaurants), and an annual slope change of -2.65% (95% CI -5.11 to -0.13; P=0.04) after 1 January 2010 (ban on smoking in bars serving food). The analysis for all births gave similar results: a step change of -3.18% (95% CI -5.38 to -0.94; P<0.01) on 1 January 2007, and an annual slope change of -3.50% (95% CI -6.35 to -0.57; P=0.02) after 1 January 2010. These changes could not be explained by personal factors (infant sex, maternal age, parity, socioeconomic status, national origin, level of urbanization); time related factors (underlying trends, month of the year, day of the week); or population related factors (public holidays, influenza epidemics, and short term changes in apparent temperature and particulate air pollution).

Conclusions Our study shows a consistent pattern of reduction in the risk of preterm delivery with successive population interventions to restrict smoking. This finding is not definitive but it supports the notion that smoking bans have public health benefits from early life.

Introduction

It is well established that active maternal smoking during pregnancy impairs fetal growth[1 2] and shortens gestation.[2-4] Moreover, second-hand smoke has also been found to affect birth outcomes.[2 3 5-13] A meta-analysis on passive smoking during pregnancy and fetal health estimated that exposure of non-smoking pregnant women to second-hand smoke reduces mean birth weight by 33 g or more, and increases the risk of a birth weight below 2,500 g by 22%.[11] A clear effect on gestational length was not found,[11] although many studies did report a significant association between second-hand smoke and premature birth.[2 3 5-10 12 13] A large body of evidence suggests that low birth weight (<2,500 g)[14 15] and premature birth (<37 weeks gestation)[16-18] are important risk factors for morbidity and mortality in childhood[15 17 18] and in adulthood.[14 16] Interventions to reduce exposure to second-hand smoke have been found to reduce the incidence of cardiovascular diseases,[19-23] but only a few studies have examined the effect on pregnancy outcomes.[7 24 25] To our knowledge, only two studies have investigated the impact of a smoking ban on birth weight and preterm birth.[24 25] However, one study did not take into account time trends,[24] and both studies examined the effect on outcomes of only a single change in legislation.

In Belgium, smoke-free legislation was implemented in different phases.[26] The first phase, implemented on 1 January 2006, required all public places and workplaces, except for the catering industry, to be smoke-free. The legislative ban on smoking in restaurants was introduced on 1 January 2007, while for bars serving food, smoke-free legislation was implemented on 1 January 2010. These successive steps in legislation gave us the opportunity to investigate possible stepwise changes in preterm delivery.

Methods

Data

Data on births in Flanders during the period 2002–2011 were obtained from the Study Centre for Perinatal Epidemiology (SPE). Flanders is the Dutch speaking northern part of Belgium with about six million inhabitants, and it has 68 maternity-obstetric units, where almost all (99%) births occur.[27] For each

newborn of at least 500 g, an official and coded perinatal form is completed (most often by the midwife) which includes information on ultrasound corrected gestational age. The form is sent to the SPE, where all data are controlled by an error detection program and feedback is provided.[28] A qualitative assessment of the SPE data shows that there is less than 5% discrepancy between electronic data and data derived from medical files.[28] A unique feature of the data is that 99.8% of all births (of ≥500 g) in Flanders are registered. Data concerning education and national origin of the mother are obtained through linkage of the medical birth certificates of the SPE with official birth declarations. However, this linkage is only available until 2009.

We limited our analyses to singleton, live born infants delivered at 24–44 weeks of gestation. The primary outcomes for this study were the risks of spontaneous and overall preterm delivery. Changes in the risk of infants born small-for-gestational-age, low birth weight, and birth weight were secondary outcomes. Spontaneous deliveries were obtained by excluding those births that were indicated as having been induced because of medical or other (unknown) reasons. Preterm delivery was defined as a gestational age below 37 completed weeks. Preterm births were further classified as mild (34–36 weeks), moderate (32–33 weeks) and extreme (<32 weeks) preterm. Small-for-gestational-age was defined as a birth weight below the 10th centile for the gestational age and sex of the baby. Low birth weight was defined as below 2,500 g. Maternal age was categorized as <25, 25–34, and ≥35 years. The degree of urbanization of maternal residence was dichotomized into urban or semi-urban versus rural municipalities.[29] Maternal residence was also used to create three classes of socioeconomic status at the municipality level, based on salary level, economic activity, degree of unemployment, and equipment level (facilities) of houses (such as percentage with central heating).[30]

Data on influenza epidemics in Belgium were provided by the National Influenza Center.[31] This center collects information on circulating influenza viruses, whereas the Unit of Health Services Research of the Institute of Public Health collects data on visit rates for influenza-like illnesses from a representative network of general physicians. Influenza epidemics are defined as a consultation rate above the epidemic threshold (138/100,000 inhabitants).

Mean daily temperature and relative humidity data were provided by the Belgian Royal Meteorological Institute and were used to calculate mean apparent temperature, an index of human discomfort.[32] We used data from a central and representative station in Uccle (Brussels, Belgium), because the region of Flanders is uniform for temperature, as a result of small altitudinal and latitudinal gradients. Data on daily mean particulate matter (PM_{10}) were obtained from the Belgian Interregional Environment Agency (IRCEL), which monitors ambient air quality with a dense network of automatic monitoring sites.[33]

Because data on education and national origin of mothers were only available until 2009, they were used in a sensitivity analysis. Education level was defined as low (lower secondary education or less), medium (secondary education completed), or high (higher education). National origin of the mother was categorized as European, Asian, Middle Eastern, African, North American, South American, or Australian.

The ethical committee of Hasselt University approved the study.

Statistical analyses

We explored the time trend in preterm delivery by using a smoothing spline on monthly rates with 10 degrees of freedom in the SAS GAM procedure. For the main analysis, based on individual level data, we used logistic regression models to test the hypothesis that there were changes in spontaneous and overall preterm delivery immediately after the introduction of the different phases of smoke-free legislation. The minimum duration of pre-legislative data was four years (that is, before the first phase of the legislation, on 1 January 2006) and the minimum amount of post-legislative data was two years (that is, after the start of the third phase of the legislation, on 1 January 2010).

The models allowed for an underlying trend throughout the study period and were adjusted for infant sex, maternal age, parity, socioeconomic status (municipality level or individual level (in sensitivity analysis)), national origin (in sensitivity analysis), level of urbanization of maternal residence, month of the year, day of the week, public holidays, influenza epidemics, apparent temperature, and PM_{10} levels. For apparent temperature and for PM_{10}, we calculated the average

exposure on the day of delivery and the day before, and we allowed for non-linear associations with preterm birth by using natural cubic splines.[34] We used four degrees of freedom for apparent temperature and two degrees of freedom for PM_{10}.

The immediate effect of smoke-free legislation was modelled as a step function (step change), including a binary indicator variable which takes a value of 1 when the ban is present and 0 otherwise, while the gradual effects were studied with an interaction term between the indicator variable and time (trend or slope change). We started by examining the effect of the three legislation phases by using separate models for each phase, including either the step change or the slope change into the model. Then, we entered the three phases in the same model: in a first model we included only the step changes of the three phases, and in a second model we included only the slope changes. Finally, we started with a full model including the three step changes and the three slope changes and in subsequent models we removed the least significant factors one at a time. From the above models, we selected the best performing model based on the Akaike Information Criterion statistic.

We also tested interaction terms to examine potential differences in the immediate and gradual effects of the smoking bans between subgroups, after also allowing for different pre-legislation trends. The beta coefficients derived from the logistic regression models for the step and slope changes were converted into percentage changes using the formula $100\times(\exp(\beta)-1)$. In sensitivity analyses, models were additionally adjusted for education and national origin of the mother (both available until 2009).

In a secondary analysis, we investigated the risk of small-for-gestational-age and low birth weight by using the same methods as described above, and we analyzed average birth weight as a continuous variable by using linear regression models. All the analyses were performed by using SAS version 9.2 (SAS Institute, Cary, North Carolina, USA).

Results

There were 631,794 registered deliveries in Flanders during the study period (2002–2011). We excluded 24,917 (3.9%) births which did not satisfy the inclusion criteria (multiple births, stillbirths, or deliveries before 24 weeks or after 44 weeks). Among the remaining 606,877 births, 448,520 (73.9%) were spontaneous deliveries. Detailed characteristics of the study population are presented in the supplementary Table S1. Characteristics for spontaneous and overall deliveries are similar. Among the spontaneous births, 51.4% were boys, median (10–90th centile) maternal age was 29.5 (23.6–35.8) years, and median birth order was 2 (1–3). Table 1 provides details of maternal age, preterm birth, birth weight, small-for-gestational-age, and low birth weight by year. During the study period, a total of 32,123 (7.2%) spontaneous deliveries occurred before 37 weeks of gestation. Of these, 25,010 (77.9%) were mild preterm, 3,518 (10.9%) were moderate preterm, and 3,595 (11.2%) were extreme preterm. A total of 44,225 (9.9%) spontaneous births were small-for-gestational-age, and 23,570 (5.3%) were low birth weight. Median (10–90th centile) birth weight was 3,370 (2,835–3,950) g among full term infants and 2,520 (1,490–3,150) g among preterm infants.

Figure 1 shows the smoothed curve of the crude percentage of spontaneous preterm deliveries by month of birth as an exploratory analysis. From the figure, the preterm birth rate was relatively stable before the first smoke-free legislation, followed by a decline in the year after the introduction of the first phase of the legislation (workplace). The decline persisted after the second smoking ban (restaurants), although this was partially reversed in the first months of 2008. A second sharp decline can be noticed in 2010, coinciding with the third phase of the legislation (bars serving food).

We further studied the impact of the smoking bans by using logistic regression models. Table 2 shows the immediate (step) and gradual (slope) changes in preterm birth risk following the introduction of the different phases of smoke-free legislation, after adjustment for the potential confounders. We did not find effect modification by personal characteristics (infant sex, maternal age, parity), so final models did not include interactions terms with these variables. There was no significant baseline trend in the risk of spontaneous preterm delivery (-0.16%

(95% CI -1.18 to 0.86) per year), whereas the underlying trend for overall preterm deliveries increased (0.99% (95% CI 0.03 to 1.96) per year). Although Table 2 shows reduced risks after each of the three phases of smoke-free legislation, a comparison of models indicates that the second and third legislation phases (ban in restaurants and in bars serving food respectively) were followed by the largest changes in preterm birth. The model producing the best fit consisted of a step change on 1 January 2007 and a slope change after 1 January 2010 (Table 2). The second legislation phase was followed by a step change in the risk of spontaneous preterm delivery of -3.13% (95% CI -4.37 to -1.87; P<0.01) on 1 January 2007, whereas the third phase was followed by an annual slope change of -2.65% (95% CI -5.11 to -0.13; P=0.04) after 1 January 2010. The analysis for overall preterm delivery showed a step change in the risk of -3.18% (95% CI -5.38 to -0.94; P<0.01) on 1 January 2007, and an annual slope change in the risk of -3.50% (95% CI -6.35 to -0.57; P=0.02) after 1 January 2010. To put this in perspective, these changes correspond to a reduction of six preterm births per 1,000 deliveries over the five study years (after 2007).

Adding data on education and national origin of the mother (available until 2009) to the final model produced similar estimates for the step change in 2007 for spontaneous preterm delivery (-1.98% (95% CI -3.40 to -0.54; P<0.01)) as well as for overall preterm delivery (-3.23% (95% CI -5.62 to -0.77; P=0.01)). We did not observe significant effects of the smoking bans on the risk of low birth weight or small-for-gestational-age in the population, nor on average birth weight.

Table 1. Characteristics of study population by year, Flanders, 2002–2011. Values are medians (10–90th percentiles) unless stated otherwise

	Total births					All preterm births		Mild preterm		Moderate preterm		Extreme preterm	
Year	No of births	Maternal age (years)	Birth weight (g)	% SGA	% LBW	% Rate	Birth weight (g)	% Rate	Birth weight (g)	% Rate	Birth weight (g)	% Rate	Birth weight (g)
Spontaneous delivery													
2002	38,658	29.3 (23.3–35.3)	3,320 (2,690–3,910)	10.98	5.63	7.41	2,510 (1,480–3,170)	5.81	2,650 (2,120–3,230)	0.79	1,985 (1,390–2,480)	0.82	1,220 (750–1,795)
2003	38,990	29.4 (23.3–35.4)	3,318 (2,690–3,910)	10.49	5.69	7.42	2,520 (1,535–3,130)	5.90	2,650 (2,100–3,190)	0.78	1,940 (1,440–2,460)	0.74	1,195 (740–1,793)
2004	42,077	29.4 (23.5–35.6)	3,330 (2,705–3,920)	9.92	5.50	7.52	2,550 (1,495–3,180)	5.83	2,680 (2,110–3,240)	0.79	1,990 (1,490–2,485)	0.89	1,250 (732–1,800)
2005	43,290	29.4 (23.6–35.7)	3,330 (2,705–3,920)	10.15	5.39	7.38	2,520 (1,470–3,170)	5.80	2,655 (2,090–3,250)	0.76	1,963 (1,483–2,435)	0.82	1,160 (715–1,700)
2006	45,003	29.4 (23.6–35.8)	3,330 (2,700–3,925)	9.82	5.32	7.36	2,540 (1,530–3,140)	5.74	2,660 (2,100–3,200)	0.85	1,940 (1,475–2,400)	0.76	1,250 (720–1,735)
2007	46,276	29.4 (23.6–35.8)	3,330 (2,720–3,920)	9.83	5.12	6.93	2,520 (1,511–3,145)	5.37	2,655 (2,140–3,200)	0.79	1,995 (1,490–2,450)	0.77	1,210 (740–1,775)
2008	47,893	29.5 (23.6–35.9)	3,345 (2,720–3,940)	9.24	5.22	7.15	2,510 (1,510–3,130)	5.58	2,650 (2,110–3,200)	0.78	1,990 (1,490–2,400)	0.79	1,250 (712–1,850)
2009	48,237	29.5 (23.6–35.9)	3,345 (2,720–3,940)	9.69	5.15	6.98	2,515 (1,480–3,150)	5.38	2,660 (2,120–3,220)	0.82	1,984 (1,380–2,400)	0.78	1,240 (750–1,795)
2010	49,166	29.7 (23.8–35.9)	3,350 (2,730–3,940)	9.24	4.85	6.84	2,530 (1,470–3,135)	5.28	2,655 (2,120–3,200)	0.76	1,980 (1,470–2,470)	0.81	1,220 (705–1,770)
2011	48,930	29.7 (23.8–36.0)	3,350 (2,730–3,940)	9.62	4.89	6.80	2,510 (1,450–3,145)	5.26	2,660 (2,120–3,220)	0.72	1,963 (1,405–2,418)	0.82	1,240 (720–1,765)
Total	448,520	29.5 (23.6–35.8)	3,335 (2,710–3,930)	9.86	5.26	7.16	2,520 (1,490–3,150)	5.58	2,660 (2,115–3,215)	0.78	1,975 (1,448–2,430)	0.80	1,220 (730–1,780)

Table 1. (continued)

	Total births					All preterm births		Mild preterm		Moderate preterm		Extreme preterm	
Year	No of births	Maternal age (years)	Birth weight (g)	% SGA	% LBW	% Rate	Birth weight (g)	% Rate	Birth weight (g)	% Rate	Birth weight (g)	% Rate	Birth weight (g)
Overall delivery													
2002	55,275	29.3 (23.2–35.3)	3,350 (2,730–3,950)	10.74	4.91	5.99	2,520 (1,540–3,180)	4.74	2,650 (2,100–3,250)	0.59	1,970 (1,390–2,460)	0.61	1,220 (750–1,790)
2003	55,639	29.3 (23.2–35.4)	3,350 (2,720–3,950)	10.34	4.89	5.98	2,530 (1,600–3,140)	4.84	2,640 (2,090–3,200)	0.59	1,940 (1,440–2,460)	0.55	1,200 (740–1,790)
2004	58,041	29.4 (23.4–35.7)	3,360 (2,740–3,960)	9.90	4.87	6.26	2,555 (1,540–3,180)	4.96	2,680 (2,100–3,240)	0.62	1,990 (1,490–2,493)	0.68	1,250 (730–1,800)
2005	59,368	29.4 (23.5–35.7)	3,360 (2,740–3,960)	10.08	4.73	6.19	2,535 (1,510–3,180)	4.96	2,660 (2,080–3,260)	0.61	1,970 (1,490–2,450)	0.62	1,160 (713–1,700)
2006	60,871	29.4 (23.5–35.8)	3,360 (2,730–3,960)	9.81	4.73	6.16	2,540 (1,560–3,155)	4.90	2,660 (2,090–3,210)	0.66	1,935 (1,470–2,400)	0.60	1,260 (720–1,740)
2007	61,847	29.4 (23.5–35.9)	3,360 (2,740–3,960)	9.77	4.64	5.86	2,520 (1,560–3,145)	4.61	2,650 (2,120–3,200)	0.64	1,990 (1,480–2,430)	0.61	1,210 (740–1,770)
2008	63,999	29.5 (23.5–36.0)	3,370 (2,750–3,980)	9.30	4.69	6.11	2,520 (1,550–3,145)	4.84	2,650 (2,100–3,210)	0.63	1,990 (1,490–2,410)	0.63	1,255 (710–1,850)
2009	63,415	29.5 (23.5–36.0)	3,370 (2,740–3,975)	9.71	4.74	6.07	2,520 (1,528–3,150)	4.77	2,655 (2,090–3,220)	0.67	1,980 (1,385–2,400)	0.63	1,240 (750–1,798)
2010	64,372	29.6 (23.7–36.0)	3,375 (2,750–3,980)	9.43	4.59	5.98	2,530 (1,520–3,135)	4.69	2,650 (2,090–3,195)	0.65	1,975 (1,470–2,465)	0.64	1,220 (705–1,770)
2011	64,050	29.7 (23.8–36.0)	3,370 (2,750–3,980)	9.65	4.52	5.88	2,510 (1,490–3,155)	4.62	2,650 (2,090–3,220)	0.61	1,955 (1,400–2,408)	0.65	1,240 (712–1,765)
Total	606,877	29.5 (23.5–35.8)	3,360 (2,740–3,965)	9.85	4.73	6.04	2,530 (1,540–3,155)	4.79	2,650 (2,100–3,220)	0.63	1,970 (1,450–2,430)	0.62	1,220 (725–1,780)

Abbreviations: SGA, small-for-gestational-age; LBW, low birth weight (<2,500 g)

Preterm categories: mild = 34–36 weeks gestation, moderate = 32–33 weeks, extreme = <32 weeks

Parity was constant over the study period: median (10–90th centile) = 2 (1–3)

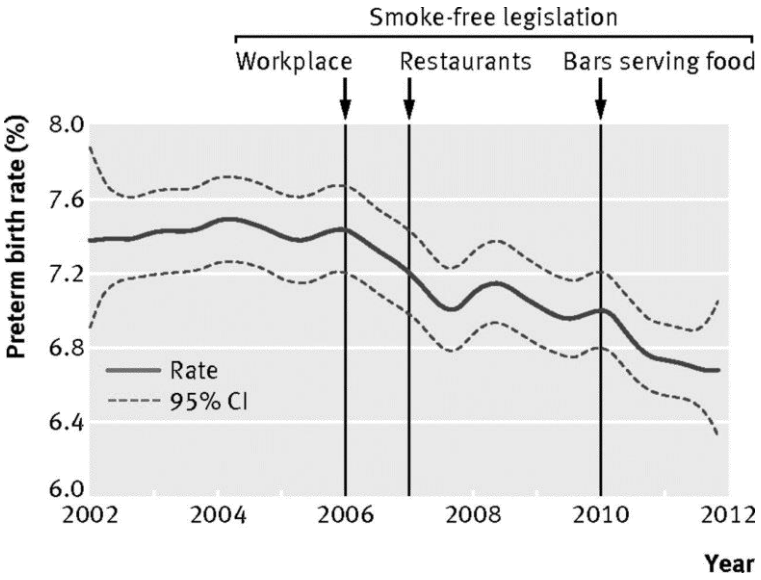

Figure 1. Time trend in rate of spontaneous preterm deliveries (with 95% CI) in Flanders, 2002–2011, with vertical lines indicating stepwise implementation of smoke-free legislation

Table 2. Percentage change in risk of preterm delivery in association with the successive implementation of public smoking bans. Values are percentage change (95% CI)

	Legislation 2006		Legislation 2007		Legislation 2010	
Model	**Step change‡**	**Slope change§**	**Step change‡**	**Slope change§**	**Step change‡**	**Slope change§**
Spontaneous preterm delivery						
2006†	-3.24 (-4.40 to -2.07)*	-1.85 (-2.42 to -1.28)*	N/A	N/A	N/A	N/A
2007†	N/A	N/A	-3.69 (-4.81 to -2.55)*	-2.16 (-2.85 to -1.46)*	N/A	N/A
2010†	N/A	N/A	N/A	N/A	-3.36 (-4.73 to -1.98)*	-5.17 (-7.36 to -2.94)*
Final	–	–	-3.13 (-4.37 to -1.87)*	–	–	-2.65 (-5.11 to -0.13)*
Overall preterm delivery						
2006†	-0.59 (-2.63 to 1.49)	-1.95 (-3.50 to -0.37)*	N/A	N/A	N/A	N/A
2007†	N/A	N/A	-2.28 (-4.37 to -0.15)*	-1.42 (-2.87 to 0.05)**	N/A	N/A
2010†	N/A	N/A	N/A	N/A	-1.24 (-3.05 to 0.60)	-2.10 (-4.82 to 0.69)
Final	–	–	-3.18 (-5.38 to -0.94)*	–	–	-3.50 (-6.35 to -0.57)*

Percentage change based on odds ratios derived from logistic regression models and adjusted for underlying trend, newborn sex, maternal age, parity, socioeconomic status, urbanization, calendar month, day of the week, public holidays, influenza epidemics, and short term changes in apparent temperature and particulate air pollution (PM_{10}). Final models were obtained by including the three step changes and the three slope changes in one model and then removing the least significant factors one at a time.

*P<0.05; **P<0.1

†Single-legislation models including either the step change or the slope change into the model

‡Step change at 1 January 2006 (legislation 2006), 1 January 2007 (legislation 2007), or 1 January 2010 (legislation 2010)

§Slope change after 1 January 2006 (legislation 2006), 1 January 2007 (legislation 2007), or 1 January 2010 (legislation 2010)

N/A = Not applicable

Discussion

We found significant reductions in the rate of preterm births after the implementation of different types of smoking bans, whereas no such decrease was evident in the years or months before these bans. Our results confirm those from a recent study.[25] Given that even a mild reduction in gestational age has been linked to adverse health outcomes in early and later life, our study has important public health implications. Indeed, a Swedish study found that, even among those born late preterm (34–36 weeks), preterm birth was associated with a 31% (95% CI 13 to 50) increase in mortality in young adulthood.[16]

As smoke-free legislation in Belgium was implemented in different phases, we were able to demonstrate a consistent pattern of changes in preterm delivery with stepwise reductions over the different enforcements. Hill proposed both internal and external consistency as one of the most important criteria of causality.[35] Although a single epidemiological study cannot prove causality, we believe that one strength of our study is that the changes showed an internally consistent pattern, with rates of preterm deliveries decreasing after each of the three smoking bans. The smoking ban at work was followed by a less marked reduction in preterm deliveries than the later ban in restaurants. It might be that the implementation of non-smoking regulation at work took place more gradually than the other bans, thus perhaps explaining why the effects of the latter were stronger (or more obvious in the analysis).

Interpretation of results

This study must be viewed as an investigation of the possible impact of a "population intervention" rather than an investigation of changes in individual behavior. It is possible that unmeasured confounders were responsible for the observed changes. Nevertheless, it is hard to conceive of a factor that could change the population risk of preterm births after the introduction of the different successive smoking bans. We adjusted for many potential confounders both at the individual level, including maternal education and national origin, and at the population level, including potential short term changes in air pollution and influenza epidemics.

It is unlikely that our observations could be explained by abrupt changes in therapeutic strategies coinciding with the smoking bans. Nevertheless, we collected data on the prescription of atosiban and on cervical cerclage treatment from a social security organization covering 42% of the population. Atosiban is an inhibitor of oxytocin and vasopressin and is specifically used to halt premature labor. Cervical cerclage is used for the treatment of cervical incompetence, a condition where the cervix has become slightly open and there is a risk of miscarriage. There were no substantial changes in the use of either treatment during the study period: the number of women given atosiban varied from 59 per 1000 deliveries (846 prescriptions per 1,000 deliveries) in 2005 to 63 per 1,000 (753 prescriptions per 1,000 deliveries) in 2011, and the number of cervical cerclages varied from 11 per 1,000 deliveries in 2002 to 8 per 1,000 deliveries in 2011.

One could object that the effects of the smoking bans were apparent only on the rate of preterm births, and we acknowledge that the absence of an effect on birth weight and small-for-gestational-age reduces the strength and diminishes the plausibility of our observation. Although some overlap exists in risk factors for prematurity and small-for-gestational-age, several investigators recommend treating these outcomes as distinct aetiologies.[36 37] Conversely, the observed dissociation between an effect on triggering labor and on birth weight may point to the need for further research on other potential triggering factors. Changes in birth weight and small-for-gestational-age at the population level might be a longer term reflection of detrimental effects of active or passive smoking during pregnancy while preterm delivery might have a discrete trigger component, as suggested by studies on short term effects of air pollution and temperature.[38-42]

Similar to our results, a study on the impact of the Irish workplace smoking ban on birth weight and preterm birth found a protective effect only on the latter outcome.[24] Although their analysis was limited to a comparison of rates one year before and after the ban, they even found an increase in the risk of low birth weight. Although a meta-analysis concluded that passive smoking does not affect preterm birth,[11] many studies did report a significant association between exposure to second-hand smoke and premature birth.[2 3 5-10 12 13] In a study of 389 non-smoking mothers, Jaakkola and colleagues[9] found that environmental tobacco smoke had stronger effects on preterm delivery than on low birth weight

and small-for-gestational-age – that is, a 1 µg/g increase in hair nicotine concentration was associated with an increase in adjusted odds ratio of 1.22 (95% CI 1.07 to 1.39) for preterm delivery compared with 1.06 (95% CI 0.96 to 1.17) and 1.04 (95% CI 0.92 to 1.19) for low birth weight and small-for-gestational-age.

Previous investigations of the health benefits at the population level of smoke-free legislation have focused primarily on the incidence of cardiovascular morbidity,[20] which is known to be triggered by various factors.[43] Further evidence of population health gains comes from observations of reduced asthma hospitalization by 18% after the introduction of smoke-free legislation.[44] The plausibility of the favorable effects of smoking bans rests on well-known effects of active and passive smoking based on animal and human studies.[45]

Limitations of study

The main limitation, common to most studies on smoking bans,[19 21-23 44] is that we do not have data on individual smoking status, neither active nor passive. The birth records also did not allow us to address other known risk factors for preterm birth, such as marital status, psychosocial stressors, maternal weight, occupation, and nutrition. However, the objective of our study was to describe the possible health impact of population based interventions, such as smoking bans, on preterm births, rather than to investigate the effects of personal exposures.

The observed effects may be due to reduced exposure of pregnant women to second-hand smoke at the workplace or in public places,[46] but they may also reflect an overall reduction in tobacco consumption. Indeed, smoke-free legislation may stimulate smokers to establish total smoking bans in their homes[47] and has resulted in increased smoking cessation,[48-50] also among pregnant women.[24 25 51] The Belgian Health Interview suggests that the total population prevalence of active smoking in Belgium was relatively stable from 1997 to 2004, but decreased significantly from 2004 to 2008.[52] More specifically, the percentage of female smokers in Flanders was close to 22% in 1997, 2001, and 2004, whereas in 2008 the percentage was only 17.9%. The prevalence of daily smoking among Flemish women decreased from 18.5% in 2004 to 15.3% in 2008 and the prevalence of heavy smoking (≥20 cigarettes a day) declined from 7.7% to 4.9%.

A survey in a random sample of 3017 women in Flanders between May 2008–09 indicated that 22.7% (95% CI 21.0 to 24.6) of the women were active smokers before pregnancy and 12.3% (95% CI 10.9 to 13.8) continued smoking during pregnancy.[53] Exposure to second-hand smoke during pregnancy was reported by 10.6% (95% CI 9.3 to 12.0) of the women.

Conclusion

Further proof of our population based observations could be provided in countries where smoking bans have been relaxed, as in the Netherlands.[54] However, along with the prospectively gained evidence on smoking and its detrimental effects on pregnancy complications, our study supports the public health benefit of smoking bans early in life.

Key messages

What is already known on this topic

Despite growing evidence that second-hand smoke has a negative impact on pregnancy duration and birth weight, few studies have shown a beneficial impact of smoke-free legislation on pregnancy outcomes.

What this study adds

Stepwise implementation of smoke-free legislation in Belgium allowed the demonstration of successive reductions in preterm deliveries which persisted after the implementation of the different legislation phases. The largest reductions in preterm birth coincided with the implementation of the second and the third phase of the legislation (smoking ban in restaurants and in bars serving food, respectively). Even a small relative decline in preterm deliveries can have important public health benefits.

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Supplementary material

Table S1. Characteristics of study population, presented as number (%) or as median (10-90th centile)

Characteristic	Value	Spontaneous delivery		Overall delivery	
Total population		448,520	(100.0)	606,877	(100.0)
Infant sex	Boy	230,368	(51.4)	311,620	(51.4)
Maternal age (years)	<25	70,759	(15.8)	98,419	(16.2)
	25-34	319,477	(71.2)	428,781	(70.7)
	35+	58,284	(13.0)	79,677	(13.1)
Parity	1	209,343	(46.7)	284,770	(46.9)
	2	159,510	(35.6)	210,731	(34.7)
	3	53,985	(12.0)	74,611	(12.3)
	≥4	87	(5.7)	36,765	(6.1)
Socio-economic status of municipality	Low	284,011	(63.5)	382,548	(63.2)
	Medium	79,892	(17.9)	108,134	(17.9)
	High	83,154	(18.6)	114,347	(18.9)
Maternal education	Low	42,424	(12.9)	58,743	(13.1)
	Medium	130,304	(39.6)	183,410	(40.8)
	High	156,123	(47.5)	207,563	(46.2)
National origin	European	278,697	(86.8)	384,522	(87.7)
	Asian	4,840	(1.5)	5,968	(1.4)
	Middle-Eastern	14,263	(4.4)	18,021	(4.1)
	African	21,061	(6.6)	27,391	(6.2)
	North-American	478	(0.2)	625	(0.1)
	South-American	1,514	(0.5)	1,942	(0.4)
	Australian	120	(0.0)	148	(0.0)
Urbanization	Urban/semi-urban	341,019	(76.0)	461,673	(76.1)
	Rural	107,501	(24.0)	145,204	(23.9)
Month	January	37,867	(8.4)	50,929	(8.4)
	February	34,299	(7.7)	46,524	(7.7)
	March	37,741	(8.4)	51,541	(8.5)
	April	37,154	(8.3)	50,035	(8.2)
	May	37,781	(8.4)	50,771	(8.4)
	June	37,730	(8.4)	50,975	(8.4)
	July	39,355	(8.8)	53,436	(8.8)
	August	39,571	(8.8)	53,366	(8.8)
	September	38,192	(8.5)	51,702	(8.5)
	October	37,646	(8.4)	51,397	(8.5)
	November	35,010	(7.8)	47,033	(7.8)
	December	36,174	(8.1)	49,168	(8.1)
Day of week	Sunday	53,697	(12.0)	56,313	(9.3)
	Monday	68,194	(15.2)	94,662	(15.6)
	Tuesday	71,804	(16.0)	104,938	(17.3)
	Wednesday	68,261	(15.2)	96,664	(15.9)
	Thursday	68,762	(15.3)	99,715	(16.4)
	Friday	65,440	(14.6)	97,554	(16.1)
	Saturday	52,362	(11.7)	57,031	(9.4)

Table S1. (continued)

Characteristic	Value	Spontaneous delivery		Overall delivery	
National holiday	Yes	10,277	(2.3)	11,124	(1.8)
Influenza epidemic	Yes	74,416	(16.6)	100,781	(16.6)
Apparent temperature (°C)		9.9	(-0.2–19.1)	9.9	(-0.2–19.1)
PM_{10} (μg/m^3)		24.5	(15.5–43.5)	24.5	(16.0–43.5)

Abbreviations: PM_{10}, particulate matter with a diameter less than 10 μm

Chapter 3

Impact of a stepwise introduction of smoke-free legislation on population rates of acute myocardial infarction deaths in Flanders, Belgium

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Abstract

Objective

Many studies demonstrated a decline in hospital admissions for cardiovascular diseases after the implementation of a smoking ban, but evidence for reductions in cardiovascular mortality is more limited. In Belgium, smoke-free legislation was implemented in different phases. Public places and most workplaces became smoke-free in January 2006, whereas the legislative ban on smoking in restaurants was introduced in January 2007. These successive steps in legislation gave us the opportunity to investigate possible stepwise changes in fatal acute myocardial infarction (AMI) rates.

Methods

Data on all AMI deaths of 30 years or older in Flanders (Belgium) between 2000 and 2009 (n=38,992) were used. Age-standardized AMI death rates were analyzed with segmented Poisson regression allowing for secular trends and seasonality.

Results

An immediate decrease in AMI mortality rates was observed in January 2006 (smoking ban at work). The effect was highest for women younger than 60 years (-33.8%, 95% CI -49.6 to -13.0), compared with an effect of -13.1% (95% CI -24.3 to -0.3) for male counterparts. Estimates for elderly (≥60 years) were -9.0% (95% CI -14.1 to -3.7) for men and -7.9% (95% CI -13.5 to -2.0) for women. An additional effect of the smoking ban in restaurants was observed for men older than 60 years, which showed an annual slope change of -3.8% (95% CI -6.5 to -1.0) after 1 January 2007.

Conclusions

Smoking ban interventions are associated with reductions in the population rate of myocardial mortality. Smoke-free legislation at the workplace had the highest impact on AMI mortality rates, with an additional effect of the smoking ban in restaurants for men of 60 years and above.

Introduction

The adverse health effects of both active and passive smoking are well-established, in particular for coronary heart diseases.[1] Growing evidence suggests that environmental tobacco exposure (ETS) increases cardiac risk through both chronic pathways such as atherosclerosis, and acute mechanisms such as platelet activation and endothelial dysfunction.[1 2] The effects of passive smoking have been found to be nearly as large (averaging 80% to 90%) as those of chronic active smoking.[1] In order to reduce exposure to second-hand smoke, many countries have implemented regulations that prohibit smoking in public places.

Many studies have shown that the enforcement of smoking bans is followed by a rapid reduction in cardiovascular disease rates in the general population, with most of the evidence coming from hospital admission data.[3-6] Most of these studies used data from hospital-based registries, whereas studies analyzing population-based registries are limited.[7 8] Moreover, only a few studies have examined the effect of smoke-free legislation on cardiovascular mortality and results are contradictory.[9-14] Population-based death registry data have greater population and geographical coverage and capture the large proportion of fatal events that occur before reaching the hospital.[15]

The magnitude of the effects of smoke-free legislation on cardiovascular disease rates is still uncertain, with estimates ranging from approximately 0 up to 70%.[2-6 8] Methodological issues might play an important role in the observed differences in effect estimates. The US Institute of Medicine highlighted the importance of the adjustment for secular trend,[2] and Gasparrini *et al.*[8] and Barr *et al.*[16] demonstrated the need to consider non-linear trends.

In Belgium, smoke-free legislation was implemented in different phases (http://www.tegenkanker.be/content/de-wet).[17] The first phase, implemented in January 2006, required all public places and workplaces (except for bars, cafes, restaurants, night clubs, and discos) to be smoke-free. The legislative ban on smoking in restaurants was introduced in January 2007, while for bars serving food, smoke-free legislation was implemented in January 2010. Finally, a comprehensive ban (including bars, discos, and casinos) was introduced in July 2011.

This study investigated the impact of the first two phases of the legislation on mortality from acute myocardial infarction (AMI) in Flanders, using cause of death data from 2000 to 2009. The two later legislation phases could not yet be examined because data were not available. To our knowledge, only one previous study has investigated cardiovascular effects of a smoking ban that was implemented in separate phases, thereby focusing on sudden circulatory arrest.[18] With six years of data before the first smoking ban and three years of data after the second ban, the pre- and post-legislative periods used in this analysis are relatively long compared to most other studies.[3-6] The robustness of the study findings to the model specification was evaluated by including non-linear secular trends.

Methods

Data

The Flemish Agency for Care and Health registers data on causes of mortality in Flanders, currently coded according to the 10th revision of the International Classification of Diseases (ICD-10). Flanders is the Dutch speaking Northern part of Belgium with about six million inhabitants. This study used data on all AMI deaths (ICD-10 code I21) among Flemish residents of 30 years and older during the period 2000-2009 (later data were not yet available). Data were aggregated by month, gender, 10-year age groups (30-39, 40-49, 50-59, 60-69, 70-79, 80+ years), and primary (underlying) or secondary diagnosis. Flemish population figures by gender, age and calendar year were obtained from the Standardized Procedures for Mortality Analysis (SPMA) website (http://www.wiv-isp.be/epidemio/spma), which presents data from the Federal Public Service Economy – Statistics Division. Mean daily air temperature, measured at the central and representative station of Uccle (Brussels, Belgium), was provided by the Belgian Royal Meteorological Institute. Mean daily particulate matter (PM_{10}) concentrations (spatial average for Flanders) were obtained from the Belgian Interregional Environment Agency (IRCEL). Weekly influenza rates for Belgium were provided by the National Influenza Center.[19] This center collects information on circulating influenza viruses, whereas the Unit of Health Services Research of

the Institute of Public Health collects data on consultation rates for influenza-like illnesses from a representative network of general physicians.

Statistical analysis

Annual age-standardized rates of AMI mortality were calculated by the direct method and using the European reference population.[20] To compare the periods before and after the smoking ban, monthly AMI mortality rates were analyzed by using an interrupted (segmented) Poisson regression, adjusting for seasonality and long-term trends. To account for changes in the age distribution during the study period, monthly age- and sex-standardized incidence rates were calculated, using the population distribution in the first month of the series (January 2000) as reference. In order to model the rates of AMI directly while keeping the actual number of AMI deaths as response variable, adjusted population sizes were calculated and entered in the model as an offset. To account for the difference in the number of days between months, the logarithm of the number of days was included as a predictor in the model. The model allowed for an underlying trend throughout the study period and seasonality was modelled by a Fourier series of sine and cosine terms.[21] Because an initial exploration of the form of the long-term trend suggested a linear pattern, a linear predictor for time was used to quantify the underlying downward trend in AMI mortality rates. Mean monthly values for temperature, PM_{10}, and influenza rates were included in the model as natural cubic splines. The degrees of freedom used (4 for temperature and influenza, 3 for PM_{10}) were chosen based on the Akaike and Bayesian Information Criteria (AIC and BIC).

The immediate effect of smoke-free legislation was modelled as a step function (level change), including a binary indicator variable which takes values 1 when the ban is present and 0 otherwise, while the gradual effects were studied with an interaction term between the indicator variable and time (trend or slope change). We started by examining the effect of the two legislation phases by using separate models for each phase, including both the step change and the slope change into the model. Then, we entered the two phases in the same model: in a first model we included only the step changes of the two phases, and in a second model we included only the slope changes. Finally, we started with a full model including the

two step changes and the two slope changes and we removed non-significant factors. The performance of the above models was checked by comparing AIC and BIC values.

Because several studies have found different effects among men and women and among younger and older persons,[7 9 10 14 22-25] analyses were stratified by gender and age below 60 years or 60 years or older. In a secondary analysis we used smaller age classes (30-49, 50-59, 60 to 69, 70 to 79, at least 80 years) to further investigate potential effect modification by age. Only cases with AMI as primary death cause were considered in the main analysis. In a sensitivity analysis, also deaths with AMI as secondary cause were included. In another sensitivity analysis, the assumed linearity of the underlying trend was formally tested. Because nonlinear models could be attributing some of the effect of the ban to the general secular trend, potential nonlinearity was investigated on pre-legislation data by testing the statistical significance of a quadratic trend orthogonal to the linear trend.[16] Additionally, we fitted the pre-legislation trend as a natural cubic spline with 1 to 5 degrees of freedom and compared the fit of models using AIC.

To control for multiple testing in the final models, we used the Benjamini-Hochberg procedure.[26] A false discovery rate smaller than 0.05 was considered significant. All the analyses were performed by using SAS version 9.2 (SAS Institute, Cary, North Carolina, USA).

Results

Table 1 presents the number of AMI deaths and the age-standardized deaths rates by calendar year, gender and age group. A gradual reduction in AMI mortality rates was evident over the total period studied, but in all four strata the largest (absolute) decrease in rates was observed in 2006. Whereas the CIs of the rates mostly overlap, there is no overlap in the CIs of the rates in 2005 and 2006 for men and women aged 60 or older.

Table 1. Yearly acute myocardial infarction (AMI) deaths and age-standardized death rates (cases/100,000) in Flanders

	Men <60 years			Women <60 years			Men ≥60 years			Women ≥60 years		
Year	**No.**	**Rate***	**(95% CI)**	**No.**	**Rate***	**(95% CI)**	**No.**	**Rate***	**(95% CI)**	**No.**	**Rate***	**(95% CI)**
2000	396	33.9	(30.5 to 37.2)	93	8.0	(6.4 to 9.7)	2,143	373.4	(357.6 to 389.3)	1,872	197.5	(188.6 to 206.5)
2001	372	30.9	(27.7 to 34.0)	92	7.9	(6.3 to 9.5)	2,125	358.9	(343.6 to 374.1)	1,822	188.7	(180.0 to 197.3)
2002	393	31.9	(28.8 to 35.1)	93	7.7	(6.2 to 9.3)	2,067	340.6	(325.9 to 355.3)	1,847	185.3	(176.8 to 193.7)
2003	361	28.8	(25.8 to 31.7)	109	8.9	(7.2 to 10.6)	2,028	326.3	(312.1 to 340.5)	1,735	168.9	(160.9 to 176.8)
2004	319	25.0	(22.3 to 27.7)	93	7.5	(6.0 to 9.0)	1,998	310.9	(297.3 to 324.6)	1,638	154.6	(147.1 to 162.0)
2005	362	27.7	(24.9 to 30.6)	89	7.0	(5.5 to 8.5)	1,970	297.7	(284.5 to 310.8)	1,572	143.9	(136.8 to 151.1)
2006	307	23.3	(20.7 to 25.9)	57	4.5	(3.3 to 5.6)	1,760	259.8	(247.7 to 271.9)	1,378	122.7	(116.2 to 129.2)
2007	271	20.4	(17.9 to 22.8)	71	5.5	(4.2 to 6.7)	1,679	239.0	(227.5 to 250.4)	1,390	122.7	(116.3 to 129.2)
2008	241	17.9	(15.6 to 20.2)	73	5.6	(4.3 to 6.8)	1,664	230.3	(219.3 to 241.4)	1,355	115.6	(109.5 to 121.8)
2009	284	21.0	(18.6 to 23.5)	57	4.3	(3.2 to 5.5)	1,583	211.1	(200.7 to 221.5)	1,233	102.4	(96.7 to 108.1)

*Age-standardized according to the European Standard Population

Final models were obtained by removing non-significant step and slope changes from the full model (with two step changes and two slope changes). These models also had the lowest BIC values, whereas AIC showed the tendency to select models containing non-significant smoking ban effects (P>0.13) (supplementary Table S1). The step change in January 2006 was retained in the final models of all subpopulations. For men of at least 60 years, the final model also included a significant slope change in January 2007. Results of the final models are reported in Table 2. The negative linear baseline trend (before 2006) in AMI mortality rates was significant in all strata except in women younger than 60 years. Among people below 60 years, the immediate decrease in the AMI death rate in January 2006 was higher for women (-33.8%, 95% CI -49.6 to -13.0) than for men (-13.1%, 95% CI -24.3 to -0.3). Estimates for elderly were lower and were of the same magnitude for men (-9.0%, 95% CI -14.1 to -3.7) and women (-7.9%, 95% CI -13.5 to -2.0). Elderly men showed an additional annual slope change of -3.8% (95% CI -6.5 to -1.0) after the second smoking ban.

Table 2. Percentage change (95% CI) in acute myocardial infarction death rates in Flanders in association with the successive implementation of public smoking bans

Age	Gender	Annual Pre-legislation trend†	Legislation 2006 Step change‡	Legislation 2007 Slope change§
<60 years	Men	-4.4 (-6.7 to -2.0)*	-13.1 (-24.3 to -0.3)*¶	N/A
	Women	0.2 (-4.6 to 5.3)	-33.8 (-49.6 to -13.0)*¶	N/A
	Both	-4.2 (-6.3 to -2.1)*	-15.3 (-25.1 to -4.2)*¶	N/A
≥60 years	Men	-4.3 (-5.3 to -3.2)*	-9.0 (-14.1 to -3.7)*¶	-3.8 (-6.5 to -1.0)*¶
	Women	-6.0 (-7.1 to -5.0)*	-7.9 (-13.5 to -2.0)*¶	N/A
	Both	-4.8 (-5.6 to -4.1)*	-8.3 (-12.2 to -4.3)*¶	-2.9 (-4.9 to -0.8)*¶

Percentage change derived from Poisson regression models and adjusted for population size, number of days, underlying trend, seasonality, temperature, PM_{10}, and influenza rates. Final models were obtained by removing non-significant factors from the full model (including the two step changes and the two slope changes).

*P<0.05

†Annual change relative to January 2000

‡Step change at 1 January 2006 (smoking ban at the workplace)

§Slope change after 1 January 2007 (smoking ban in restaurants)

¶False discovery rate <0.05 (Benjamini-Hochberg on 8 P-values)

N/A = Not applicable

Observed AMI rates and the estimated temporal trend are depicted in Figure 1. Overall, from January 2006 to December 2009, the model predicts 1,715 fewer AMI deaths in Flanders associated with the implementation of smoke-free legislation.

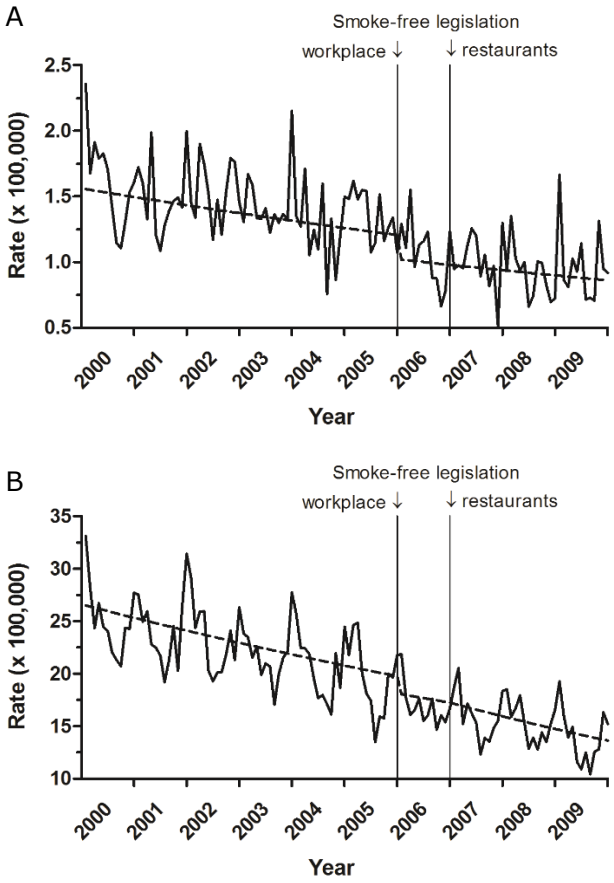

Figure 1. Observed acute myocardial infarction death rates (*solid lines*) and the estimated temporal trend (*dashed line*) among persons under 60 years of age (A) and persons aged at least 60 years (B), Flanders, 2000-2009. The step in the temporal trend at the first *vertical line* is the acute effect of the smoking ban at the workplace (A and B), and the slope change after the second *vertical line* is the gradual effect of the smoking ban in restaurants (B).

The analysis on more narrow age categories suggests that the immediate effect of the workplace smoking is highest for the youngest group (30-49 years), but estimates show an increase with age going from the middle (60-69 years) to the highest age category (80+ years). The sensitivity analysis showed that the inclusion of cases with AMI as secondary death cause produced similar results with slightly lower effect estimates. For men below 60 years, the step change in 2006 was no longer significant (P=0.12). In the analyses testing the potential nonlinear curvature of the secular trend, no significant quadratic terms were found (P>0.21) and models with a linear pre-legislation trend showed the best fit.

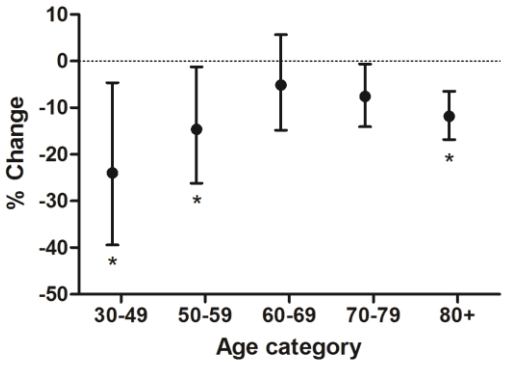

Figure 2. Immediate effect of the smoking ban at the workplace (January 2006) on acute myocardial infarction death rates in different age groups. Percentage change and 95% CIs derived from Poisson regression models and adjusted for population size, number of days, underlying trend, seasonality, temperature, particulate air pollution (PM_{10}), and influenza rates. *False discovery rate <0.05 (Benjamini-Hochberg on 5 P-values)

Discussion

This study, based on nearly 40,000 AMI deaths in Flanders, showed significant reductions in AMI death rates after the introduction of national-level smoke-free legislation. Significance of estimates remained after Benjamini-Hochberg correction for multiple testing. The effects were more pronounced after the first step of the legislation (smoking ban in workplaces and public places). Our findings are compatible with a study on out-of-hospital sudden circulatory arrest (SCA) in South-Limburg (a region in the Netherlands). Based on 2,305 cases, the authors found a significant decrease in the incidence of SCA after the workplace smoking ban in 2004, but no further decrease after the extension of the ban to the

hospitality sector in 2008.[18] Whereas they found a gradual decrease in SCA incidence (annual reduction of 6.8%) after the workplace smoking ban, we observed an immediate reduction (step change). In addition, we found some evidence for a gradual effect after the smoking ban in restaurants, but only for elderly men. However, the short time period (twelve months) between the two phases of the legislation makes it difficult to separate their effects due to potential postponed effects of the first legislation.

Although two reviews suggest that impacts of smoke-free legislation could increase over time,[3 5] some other studies[13 22 24] did not observe significant gradual effects additional to the immediate effects. Strong evidence that smoking ban effects are immediate rather than gradually is provided by the most recent meta-analysis of 45 studies that demonstrated that smoking ban effects on cardiovascular, cerebrovascular, and respiratory diseases did not increase with a longer follow-up period.[6]

The magnitude of the overall reduction in AMI mortality rates associated with the 2006 legislation found in this study (-15.3% among people aged under 60 years and -8.3% among people aged 60 years or older) is comparable with the 10% to 17% decrease in AMI hospital admissions rate estimated by four meta-analyses.[3-6] The estimated effect for women younger than 60 years in this study, however, is considerably higher (33.8%). A greater effect in younger individuals was also found in three Italian studies[7 22 25] and is plausible because banning smoking at workplaces mainly affects the working population and appears to encourage smoking cessation particularly in younger smokers.[27] The age of 60 years used in the analysis is expected to be the most realistic cut-off value to separate the working from the non-working population. Although the official retirement age in Belgium is 65 years, the median age at which people withdraw from the labor force is around 7 years below the official age.[28] Studies in Scotland, England and Spain found greater effects among elderly (above 60 or 65 years).[9 14 23 24] Although we found much larger smoking ban effects among the youngest ages, our secondary analysis suggested a higher impact in the oldest age categories compared to the group of 60-69 years. The elderly are generally considered to be a susceptible segment of the population. Their higher prevalence of cardio-respiratory conditions, together with age-related declines in physiological reserves

or homeostatic balances, makes them vulnerable to the effects of triggers such as air pollution and passive smoking.[29]

Our study suggests that the reduction in AMI mortality rates is greater among women than among men, which is compatible with findings from other studies.[9 10 23] This might be explained by a stronger decrease in exposure among women, as suggested by the greater post-ban reduction in serum cotinine levels in non-smoking women (47%) compared with men (37%) in Scotland.[23] Another reason might be the higher relative risk for AMI associated with smoking in women compared with men.[5] Tobacco smoke may have an anti-estrogenic effect, particularly in young, premenopausal women who would otherwise benefit from estrogen's cardio-protective role.[30] However, the Rome, England and Italian 4 regions studies found greater effects among men.[7 24 25]

Studies to date have been heterogeneous in their designs, target populations, statistical analyses, choices of control groups, and types of smoking bans investigated.[16] Misspecification of the underlying long-term trend and other issues such as changes in population size, sampling variations, different lengths of follow up, changes in active smoking and differences in the prevalence of active and passive smoking have been raised to explain the differences in the effect estimates between studies.[3 5 8 24] Gasparrini *et al.*[8] and Barr *et al.*[16] argue that the beneficial effect of smoking bans may be overestimated because nonlinearity of trends in declining cardiovascular morbidity is not adequately taken into account in many studies. Therefore, the potential nonlinear curvature of the underlying trend was formally tested in a sensitivity analysis. The validity of the linear models used in the main analysis was confirmed.

Can the reduction in AMI rates be considered a causal consequence of the smoking ban? A population intervention must be interpreted at the population level and not at the individual level. As this was an ecological study, it is possible that unmeasured confounders were responsible for the observed effects. Nevertheless, it is hard to conceive of a factor that could change the population risk of AMI mortality at the moment of the introduction of smoke-free legislation and it is unlikely that the study findings could be explained by abrupt changes in therapeutic strategies coinciding with the smoking bans. Moreover, the plausibility of the favorable effects of smoking bans rests on well-known effects of active and

passive smoking based on animal and human studies.[31] Indeed, tobacco smoke consists of large amounts of particulate matter. Lung[32] and systemic inflammation, prothrombotic reactions,[33] modulations in heart rate variability[34] and blood pressure[35] are mechanisms which explain the association between AMI and particulate air pollution[29] at concentrations which are much lower than exposure to second-hand smoke.

Limitations of this study include the absence of a comparison with a nearby control population without smoking ban and the lack of data on individual smoking status neither active nor passive. The observed effects may include reduced exposure to ETS[36] as well as direct health benefits from reduced tobacco consumption. Indeed, smoke-free legislation may stimulate smokers to establish total smoking bans in their homes[37] and has resulted in increased smoking cessation.[27] The Belgian Health Interview suggests that the total population prevalence of active smoking in Belgium was relatively stable from 1997 to 2004, but decreased significantly from 2004 to 2008 (http://www.wiv-isp.be/epidemio/hisia/index.htm). More specifically, the percentage of female smokers in Flanders was close to 22% in 1997, 2001 and 2004, while in 2008 the percentage was only 17.9%. The prevalence of daily smoking among Flemish females reduced from 18.5% in 2004 to 15.3% in 2008 and the prevalence of heavy smoking (20 cigarettes or more per day) reduced from 7.7% to 4.9%.

Strengths of this study include the opportunity to investigate a stepwise implementation of smoke-free legislation, the large population size and the relatively long pre- and post-legislation period. Several earlier studies used a "before and after" design and might have been unable to fully account for underlying trends in AMI rates. Declining time trends, as observed in this study, might be caused by the concomitant effect of other time-varying factors, like changes in the distribution of known risk factors, health care improvements and development of diagnostic criteria.

Conclusion

This study found population changes in fatal AMI rates after the introduction of smoke-free legislation in Belgium. Given that coronary heart disease is the single most common cause of death and morbidity worldwide, even a small reduction in the incidence of AMI is of great benefit for public health. Our study indicates that 1,715 AMI deaths were likely prevented in Flanders from January 2006 to December 2009. These findings add to the evidence that smoking bans improve population health and, knowing that only 16% of the world's population is currently covered by comprehensive smoke-free laws,[38] provide further support for the introduction of smoke-free legislation worldwide.

Key messages

What is already known about this subject?

Despite the large number of studies showing a decrease in hospital admissions for cardiovascular diseases after the implementation of a smoking ban, evidence for reductions in fatal acute myocardial infarction (AMI) is less abundant.

What does this study add?

The stepwise implementation of smoke-free legislation in Belgium allowed the investigation of potential successive reductions in AMI mortality rates. Significant immediate decreases in rates of fatal AMI were observed after the first step of the legislation (smoking ban in workplaces and public places). For men of at least 60 years, an additional effect of the smoking ban in restaurants was found.

How might this impact on clinical practice?

Given that AMI is the leading cause of death and a major economic burden in most countries, our findings have important public health implications and provide further support for the introduction and continuation of smoke-free laws worldwide.

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Supplementary material

Table S1. Model selection for the association between myocardial infarction (AMI) death rates in Flanders and the successive implementation of public smoking bans

Model	AIC†	BIC‡	Annual pre-legislation trend§		Legislation 2006				Legislation 2007			
					Step change¶		Slope change#		Step change¶		Slope change#	
Men < 60 years												
full	768.8	835.7	-4.5	(-7.0 to -1.9)*	-2.7	(-26.2 to 28.3)	-7.2	(-41.3 to 46.8)	-6.4	(-30.1 to 25.3)	6.7	(-33.4 to 70.7)
step 2006 + slope 2006	765.4	826.8	-4.6	(-7.1 to -2.1)*	-6.6	(-19.5 to 8.4)	-3.9	(-9.4 to 2.0)		N/A		N/A
step 2007 + slope 2007	765.6	826.9	-5.1	(-7.2 to -3.0)*		N/A		N/A	-12.6	(-27.3 to 5.1)	-0.4	(-9.1 to 9.0)
step 2006 + step 2007	764.9	826.2	-4.5	(-7.0 to -2.0)*	-6.1	(-19.0 to 8.9)		N/A	-10.9	(-23.4 to 3.5)		N/A
slope 2006 + slope 2007	765.0	826.3	-4.5	(-6.9 to -2.1)*		N/A	-13.8	(-27.8 to 2.9)		N/A	13.8	(-8.9 to 42.1)
step 2006	765.2	**823.7**	-4.4	(-6.7 to -2.0)*	-13.1	(-24.3 to -0.3)*		N/A		N/A		N/A
step 2006 + slope 2007	765.8	827.2	-4.7	(-7.2 to -2.2)*	-9.1	(-20.8 to 4.4)		N/A		N/A	-4.0	(-10.4 to 2.9)
Women < 60 years												
full	594.7	661.4	-0.7	(-5.8 to 4.7)	-45.9	(-71.1 to 1.3)	14.9	(-58.3 to 216.5)	34.6	(-27.6 to 150.3)	-20.5	(-71.8 to 123.9)
step 2006 + slope 2006	593.5	654.6	-0.2	(-5.3 to 5.2)	-35.9	(-53.0 to -12.5)*	2.8	(-9.1 to 16.3)		N/A		N/A
step 2007 + slope 2007	602.1	663.3	-5.5	(-9.6 to -1.2)*		N/A		N/A	6.9	(-25.8 to 53.8)	-4.8	(-20.8 to 14.5)
step 2006 + step 2007	591.7	652.8	-1.0	(-6.1 to 4.3)	-40.7	(-57.0 to -18.2)*		N/A	26.4	(-8.8 to 75.2)		N/A
slope 2006 + slope 2007	599.4	660.5	-2.3	(-7.2 to 2.8)		N/A	-26.3	(-48.3 to 4.9)		N/A	40.6	(-10.3 to 120.4)
step 2006	591.7	**650.1**	0.2	(-4.6 to 5.3)	-33.8	(-49.6 to -13.0)*		N/A		N/A		N/A
step 2006 + slope 2007	593.7	654.8	0.1	(-5.0 to 5.4)	-34.2	(-50.3 to -12.8)*		N/A		N/A	1.3	(-12.1 to 16.9)

Table S1. (continued)

Model	AIC†	BIC‡	Annual pre-legislation trend§		Legislation 2006				Legislation 2007			
					Step change¶		Slope change#		Step change¶		Slope change#	
Men ≥ 60 years												
full	1027.8	1094.7	-4.3	(-5.3 to -3.2)*	-4.2	(-14.7 to 7.6)	-8.6	(-24.2 to 10.0)	2.6	(-8.7 to 15.3)	5.8	(-12.6 to 28.1)
step 2006 + slope 2006	1024.9	1086.3	-4.2	(-5.3 to -3.2)*	-6.9	(-12.5 to -0.9)*	-3.4	(-5.8 to -1.1)*		N/A		N/A
step 2007 + slope 2007	1032.8	1094.2	-5.1	(-6.0 to -4.2)*		N/A		N/A	-5.8	(-12.6 to 1.5)	-2.6	(-6.1 to 1.1)
step 2006 + step 2007	1028.1	1089.4	-4.4	(-5.5 to -3.3)*	-7.8	(-13.4 to -2.0)*		N/A	-6.2	(-11.8 to -0.2)*		N/A
slope 2006 + slope 2007	1025.1	1086.4	-4.4	(-5.4 to -3.3)*		N/A	-11.1	(-17.3 to -4.5)*		N/A	9.9	(0.4 to 20.3)*
step 2006	1030.1	1088.6	-4.8	(-5.8 to -3.8)*	-10.1	(-15.1 to -4.8)*		N/A		N/A		N/A
step 2006 + slope 2007	1024.9	**1086.2**	-4.3	(-5.3 to -3.2)*	-9.0	(-14.1 to -3.7)*		N/A		N/A	-3.8	(-6.5 to -1.0)*
Women ≥ 60 years												
full	959.5	1026.4	-6.1	(-7.3 to -5.0)*	-15.4	(-25.8 to -3.5)*	9.9	(-10.9 to 35.6)	9.0	(-4.3 to 24.1)	-13.9	(-30.7 to 6.8)
step 2006 + slope 2006	964.8	1026.1	-5.9	(-7.1 to -4.8)*	-7.2	(-13.4 to -0.7)*	-0.8	(-3.4 to 2.0)		N/A		N/A
step 2007 + slope 2007	967.2	1028.5	-7.2	(-8.1 to -6.2)*		N/A		N/A	7.0	(-1.5 to 16.2)	-4.3	(-8.2 to -0.3)*
step 2006 + step 2007	963.0	1024.4	-6.3	(-7.5 to -5.2)*	-9.8	(-15.8 to -3.3)*		N/A	5.2	(-1.9 to 12.8)		N/A
slope 2006 + slope 2007	969.4	1030.7	-6.6	(-7.7 to -5.5)*		N/A	-2.3	(-9.8 to 5.9)		N/A	0.5	(-9.1 to 11.0)
step 2006	963.1	**1021.6**	-6.0	(-7.1 to -5.0)*	-7.9	(-13.5 to -2.0)*		N/A		N/A		N/A
step 2006 + slope 2007	964.0	1025.3	-5.8	(-7.0 to -4.7)*	-7.5	(-13.1 to -1.4)*		N/A		N/A	-1.7	(-4.8 to 1.5)

Percentage change (95% CI) derived from Poisson regression models and adjusted for population size, number of days, underlying trend, seasonality, temperature, particulate air pollution (PM_{10}), and influenza rates. Final models (in bold) were obtained by removing non-significant factors from the full model (including the two step changes and the two slope changes).

*P<0.05

†Akaike Information Criterion

‡Bayesian Information Criterion

§Annual change relative to January 2000

¶Step change relative to December 2005 (model 2006) or relative to December 2006 (model 2007)

#Annual change relative to December 2005 (model 2006) or relative to December 2006 (model 2007)

N/A = Not applicable

Chapter 4

Ambient temperature as trigger of preterm delivery in a temperate climate

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Submitted

Abstract

Preterm birth is the leading cause of perinatal morbidity and mortality in developed countries. Findings from studies on the short-term effects of meteorological variables on preterm birth are inconclusive, especially for colder climates. Therefore, we investigated the triggering effect of temperature on the risk of preterm birth during the warm and cold season in Flanders (Belgium). We used a quasi-Poisson model on 446,110 singleton natural deliveries between 1998 and 2011. We accounted for the daily pregnancies at risk and their gestational age distribution and we allowed for delayed and non-linear temperature effects. Relative to the median value of 12.8°C, the risk of preterm birth in the warm season increased by 14.1% (95% CI 4.7 to 24.2) when minimum temperature up to 3 days before delivery (lag 0–3) exceeded the 95th percentile (17.9°C). The corresponding estimate for maximum temperature was not significant (2.6%, 95% CI -8.7 to 15.4). In the cold season, we observed a significant effect of low minimum temperature on the risk of preterm birth two days before birth (lag 2), but cumulative cold effects were not significant. Even in a temperate climate, ambient temperature might trigger preterm delivery, suggesting that pregnant women should be protected from temperature extremes.

Introduction

Preterm delivery is the primary cause of perinatal morbidity and mortality in developed countries.[1] While adverse health outcomes are most obvious for lower gestational age,[2] even late preterm births (34-36 weeks) have higher morbidity and mortality rates than full-term births.[3-5] Because many of these effects continue into childhood and even adulthood,[3] preterm birth is considered a serious public health issue with major economic implications.[6 7]

Despite extensive research and clinical efforts designed toward the reduction of preterm delivery, rates continue to rise in most regions of the world. In Europe the preterm birth rate is generally 5–9%, and in the US it has even increased to 12–13% during the last decades.[1] The underlying causes of preterm birth are poorly understood, although genetic, demographic, social-behavioral, and environmental factors all likely play a role.[1 8]

Whereas epidemiological evidence for an association between air pollution and preterm birth is accumulating,[9-11] studies on the effects of ambient temperature are limited and findings are inconclusive.[12 13] Except for the most recent, many of these studies included no or only few confounders[13] or did not take into account the seasonal heterogeneity in the pregnancies at risk for preterm birth.[14 15] Inconsistencies between studies might be related to differences in study design, statistical methods, exposure windows, temperature indicators or climate. Among the few studies conducted in colder climates,[15-18] only one has found an association between preterm birth and temperature.[17] Moreover, although studies on seasonality of birth outcomes have reported peaks of preterm birth not only in summer but also in winter,[19 20] only a limited number of studies have investigated the short-term effect of cold.[15 17 18 21 22]

We studied the association between preterm births and ambient temperature in the temperate climate of Flanders (Belgium). We investigated the impact of both heat and cold and we considered the possibility of both non-linear and lagged exposure effects.

Methods

Data

The Study Centre for Perinatal Epidemiology (SPE) supplied information on all births in Flanders during the period 1998-2011. Flanders is the Dutch speaking Northern part of Belgium with about six million inhabitants and 68 fully equipped maternity-obstetric units.[23] For each newborn of at least 500 g, an official and coded perinatal form is completed and sent to the SPE, where data are controlled by an error detection program.[24] A qualitative assessment of SPE-data shows that there is less than 5% discrepancy between electronic data and all data derived from medical files and that 99.8% of all pregnancies are registered.[24 25] Gestational age is based on the last menstrual period and is corrected with the estimation from the first ultrasound.

We limited our study population to live-born singleton births with a gestational age between 22 and 42 weeks. Induced deliveries and caesarean sections were excluded because these are less likely to be related to external factors such as temperature. Preterm births were classified as extremely to very preterm (<32 weeks) and moderate to late preterm (32-36 weeks).[26] Other available information included date of birth, infant's sex, parity, mother's age and postal code of municipality of residence at the time of birth. Information on maternal education was gathered through linkage of the medical birth certificates of the SPE with official birth declarations. This linkage, however, is only available from 1999 to 2009.

Data on daily minimum and maximum air temperature and relative humidity were provided by the Belgian Royal Meteorological Institute. We used data from a central and representative station in Uccle (Brussels), because the region of Flanders is quite uniform for temperature, due to extremely small altitudinal and latitudinal gradients: elevations range from 0 to 200 m above sea level, and the distance between the most northern and most southern part is less than 100 km.

Daily particulate matter with diameter less than 10 $\mu g/m^3$ (PM_{10}, daily average) and ozone (8-hour maximum) concentrations were obtained from the Belgian Interregional Environment Agency. A validated model provides interpolated air pollutant concentrations in 4 by 4 km grids based on the Belgian telemetric air

quality network.[27] Using these data, population-weighted average daily concentrations for Flanders were calculated. In order to account for potential confounding by influenza episodes, data on consultation rates for influenza-like illnesses in Belgium are collected from a representative network of general physicians.[28]

The research protocol was approved by the medical ethics committee of Hasselt University.

Statistical analysis

Separate analyses were done for the warm (May-September) and the cold season (October-April) and for each temperature indicator (minimum and maximum temperature). To have complete cold seasons, we excluded the first 4 months of 1998 and the last 3 months of 2011. We also excluded the last warm season (May-September 2011) in order to avoid the decrease in the denominator (i.e. the pregnancies at risk of preterm birth) at the end of the dataset. The short-term effects of temperature on the risk of preterm birth were investigated by using a quasi-Poisson model with the daily count of preterm births as outcome variable.

To account for the temporal variation in the number of pregnancies at risk of preterm birth and their gestational age distribution, we used a modification of the original pregnancies-at-risk approach,[14] which has been previously applied by Vicedo-Cabrera *et al.*:[29] daily counts of the pregnancies at risk of being preterm are used as an offset and a daily weight indicator (calculated based on the probability of giving birth conditional on the gestational age in each day) is entered into the model (natural cubic spline with 4 degrees of freedom (df) in this analysis).

We combined the quasi-Poisson model with a distributed lag non-linear model (DLNM).[30 31] The DLNM is defined through a "cross-basis" function, which allows simultaneous estimation of the non-linear exposure-response association and the non-linear effects across lags, the latter termed lag-response association. We used a natural cubic spline–natural cubic spline cross-basis. Spline knots were placed at equal spaces in the temperature range to allow enough flexibility in the two ends of the temperature distribution. The knots in the lag space were set at

equally spaced values on the log scale of lags to allow more flexible lag effects at shorter delays.[32] The maximum lag was set to 10 days. The df for temperature and lag were selected according to the Akaike information criterion for quasi-Poisson models (q-AIC),[30 33] varying the df for the exposure-response from 2 to 6 and for the lag-response from 3 to 6.

Long-term trends[25] were modelled using a natural cubic spline of the variable time allowing 1 df for every 2 years of data. To control for seasonality, we fitted a natural cubic spline of day of the season with 1 df per month. We further adjusted for potential non-linear effects of humidity, PM_{10}, and ozone, by using natural cubic splines with 3 df for the moving average of the current day and the previous day (lag 0–1). Day of the week, public holidays and influenza epidemics were controlled for by use of dummy variables. For each of the temperature indicators, the median values within the season were used as reference (centering value). Relative risks (RR) were calculated at the 95th and the 99th percentiles of the temperature indicator within the warm season and at the 5th and the 1st percentiles within the cold season. Reported estimates, computed as the overall cumulative risk accounting for the 0-10 lag period, are presented as percent change in preterm birth relative to the reference temperature.

In order to identify subpopulations that are more susceptible to the effects of hot and/or cold temperatures, we conducted stratified analyses by gestational age (<32 weeks, 32-36 weeks), gender, parity (first, higher), maternal age group (<25 years, 25-34 years, ≥35 years), maternal education (lower secondary or less, higher secondary, higher education) and the degree of urbanization of mother's residence (urban/semi-urban versus rural).[34] Because temperature may also act as a trigger for labor among term births,[16] we additionally considered early term (37-38 weeks) and late term (>38 weeks) births in the stratified analyses. The pregnancies at risk and the daily weight indicator were recalculated for each of the subpopulations.

To explore the potential confounding of air pollution, we performed a sensitivity analysis by adding one air pollution variable at a time or excluding both pollution variables from the model. We also validated whether the estimates were robust with respect to the specification of the long-term and seasonal trends by changing the flexibility of the spline function from 0.5 to 2 df for every 2 years of data and

from 0.5 to 2 df per month respectively. In a final sensitivity analysis, we excluded lag 0 from the cross-basis and included it as a separate term (natural cubic spline with 3 df) into the model. This was done because, especially for maximum temperature, lag 0 suffers from the problem that the event (birth) might precede the exposure. Since DLNM typically models delayed effects through a function, estimates for later lags are likely to be influenced by lag 0.[29]

All the analyses were performed with the statistical software R, using the "dlnm" package.[32]

Results

Data description

During the study period (May 1998 – April 2011) there were 771,796 live born singletons with a gestational age from 22 to 42 weeks in Flanders. After excluding a total of 325,686 (42.2%) caesarean sections and induced births, our study population consisted of 446,110 births, of which 25,959 (5.8%) were preterm (Table 1). The number of preterm births per day ranged from 0 to 17 in the warm season and from 0 to 15 in the cold season. In both seasons, the mean number of cases per day was around 5 and the percentage of days without cases was less than 0.5%. The majority of preterm births (92.6%) had a gestational age from 32 to 36 weeks (moderate to late preterm).

Table 1. Descriptive characteristics of the study population, Flanders (Belgium), May 1998 – April 2011

		All births		Preterm births	
Characteristic	**Value**	**No.**	**%**	**No.**	**%**
Total		446,110	100.0	25,959	100.0
Season	Warm (May-September)	191,018	42.8	10,949	42.2
	Cold (October-April)	255,092	57.2	15,010	57.8
Gestational age	<32 weeks	1,913	0.4	1,913	7.4
	32-36 weeks	24,046	5.4	24,046	92.6
	37-38 weeks	101,324	22.7	N/A	N/A
	>38 weeks	318,827	71.5	N/A	N/A
Gender	Male	228,967	51.3	14,518	55.9
	Female	217,143	48.7	11,441	44.1
Parity	First	237,810	53.3	11,577	44.6
	Higher	208,300	46.7	14,382	55.4
Maternal age	<25 years	76,083	17.1	5,444	21.0
	25-34 years	319,829	71.7	17,547	67.6
	≥35 years	50,198	11.3	2,968	11.4
Maternal education*	Low	46,611	12.5	3,339	15.2
	Medium	137,427	36.8	9,145	41.7
	High	164,519	44.1	7,978	36.4
	Missing data	24,519	6.6	1,457	6.6
Urbanization	Urban	336,822	75.5	19,649	75.7
	Rural	109,288	24.5	6,310	24.3

*From 1999 to 2009 (373,076 births and 21,919 preterm births)

N/A = Not applicable

Table 2 shows summary statistics for daily weather variables and air pollutants. In the warm season, minimum temperature ranged from 1.3 to 23.9°C and maximum temperature from 6.5 to 35.8°C. In the cold season, minimum temperature ranged from -12.3 to 16.3°C and maximum temperature from -6.0 to 28.5°C. The median PM_{10} and ozone in the warm season were 25.1 µg/m^3 and 53.1 µg/m^3 respectively, whereas the corresponding concentrations in the cold season were 27.0 µg/m^3 and 35.5 µg/m^3 respectively.

Table 2. Summary statistics of the meteorological and air pollution variables by season, Flanders (Belgium), May 1998 – April 2011

	Percentiles				
Exposure	**0th**	**25th**	**50th**	**75th**	**100th**
Warm season (May–September)					
Minimum temperature (°C)	1.3	10.5	12.8	14.8	23.9
Maximum temperature (°C)	6.5	18.0	20.6	23.7	35.8
Mean relative humidity (%)	32.1	67.3	74.6	81.5	97.7
PM_{10} (µg/m^3)	9.3	19.4	25.1	32.2	81.2
Ozone (µg/m^3)	13.2	43.7	53.1	65.3	129.9
Cold season (October–April)					
Minimum temperature (°C)	-12.3	1.1	4.5	7.7	16.3
Maximum temperature (°C)	-6.0	6.2	9.8	13.4	28.5
Mean relative humidity (%)	35.2	75.5	82.9	88.8	99.9
PM_{10} (µg/m^3)	5.2	20.0	27.0	37.3	150.5
Ozone (µg/m^3)	6.1	21.5	35.5	50.7	97.0

Abbreviations: PM_{10}, particulate matter with a diameter less than 10 µm

Warm season

The final model for minimum as well as maximum temperature in the warm season contained 2 df for temperature and 3 df for the lag structure. The 3-dimensional graph for minimum temperature shows an acute increase in the risk of preterm birth at high temperatures, which is largest at lag 0 (Figure 1A). Using maximum temperature, the increase in the preterm birth risk at high temperatures is much smaller and only appears after some lags (Figure 1B).

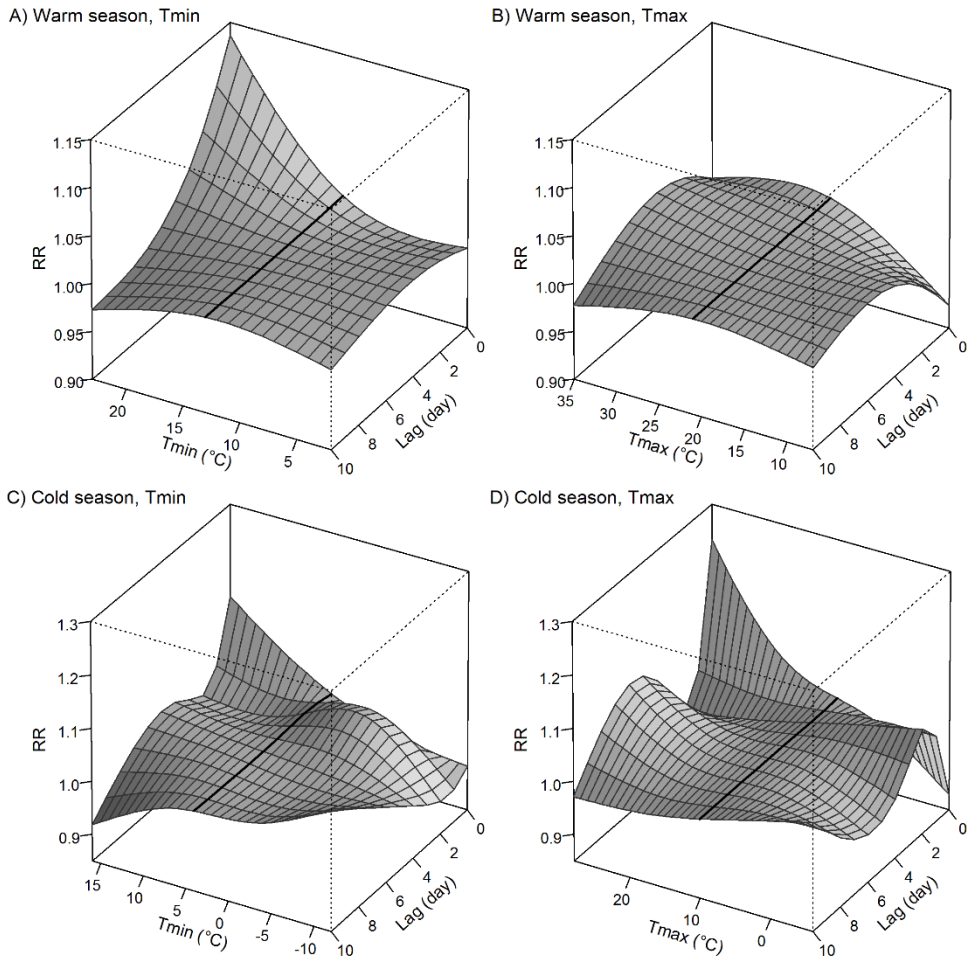

Figure 1. Relative risks (RR) of preterm birth by minimum (Tmin) and maximum temperature (Tmax) in the warm (A, B) and the cold (C, D) season. Reference values (bold lines) are median temperatures (12.8°C for Tmin and 20.6°C for Tmax in the warm season; 4.5°C for Tmin and 9.8°C for Tmax in the cold season), Flanders, Belgium, May 1998 – April 2011.

Figure 2 presents the effect of high temperature on the risk of preterm birth along the lags, contrasting the 95th and the 99th percentiles to the 50th percentile of the temperature indicator. Heat effects were significant for minimum temperature from lag 0 to lag 2 (Figure 2A), but there were no significant effects for maximum temperature (Figure 2B).

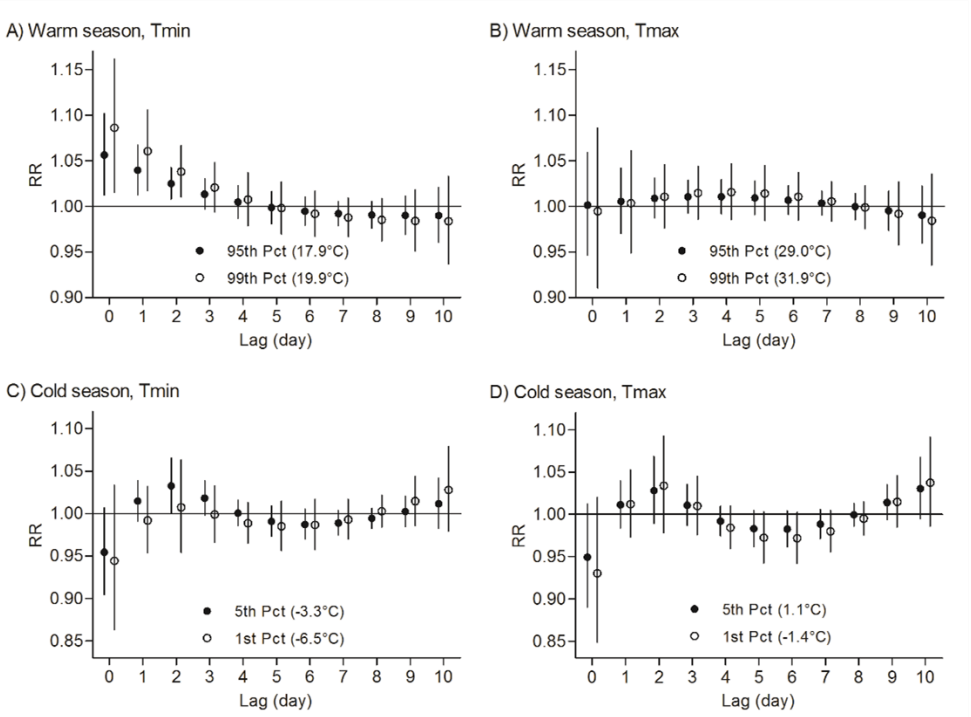

Figure 2. Lag-specific effects of minimum (Tmin) and maximum (Tmax) temperature on preterm birth, Flanders, Belgium, May 1998 – April 2011. Heat effects (warm season: A, B) are estimated at the 95th (solid circles) and the 99th percentile (open circles), relative to the median (12.8°C for Tmin and 20.6°C for Tmax). Cold effects (cold season: C, D) are estimated at the 5th (solid circles) and the 1st percentile (open circles), relative to the median (4.5°C for Tmin and 9.8°C for Tmax). The symbols are mean relative risks (RR) and the error bars are 95% CIs.

The cumulative effects of minimum temperature on preterm birth are presented in Table 3. Considering the heat effect up to 3 days before delivery (lag 0–3), the risk of preterm birth increased by 14.1% (95% CI 4.7 to 24.2) when minimum temperature increased from 12.8° (median) to 17.9°C (95th percentile) and by 22.0% (95% CI 6.4 to 39.9) when minimum temperature increased to 19.9°C (99th percentile). The corresponding estimates for maximum temperature were not significant: 2.6% (95% CI -8.7 to 15.4) for an increase from 20.6°C to 29.0°C and 2.3% (95% CI -15.0 to 23.0) for an increase from 20.6°C to 31.9°C (supplementary Table S1).

Table 3. Percent change (with 95% CI) in the risk of preterm birth associated with high minimum temperatures (95th and 99th percentile) in the warm season and low minimum temperatures (5th and 1st Percentile) in the cold season, relative to the median values of the season, Flanders, Belgium, May 1998 – April 2011

	Percent change (95% CI)†			
	WARM season (50th Pct = 12.8°C)		**COLD season (50th Pct = 4.5°C)**	
Lag (day)	**95th Pct = 17.9°C**	**99th Pct = 19.9°C**	**5th Pct = -3.3°C**	**1st Pct = -6.5°C**
0	5.6 (1.3 to 10.2)*	8.6 (1.6 to 16.2)*	-4.6 (-9.5 to 0.6)	-5.6 (-13.7 to 3.3)
0–1	9.8 (2.6 to 17.5)*	15.2 (3.4 to 28.3)*	-3.2 (-8.7 to 2.7)	-6.3 (-15.0 to 3.2)
0–2	12.5 (3.9 to 21.9)*	19.6 (5.2 to 35.9)*	0.0 (-5.8 to 6.1)	-5.6 (-14.4 to 4.0)
0–3	14.1 (4.7 to 24.2)*	22.0 (6.4 to 39.9)*	1.8 (-4.5 to 8.5)	-5.8 (-15.2 to 4.7)
0–5	14.4 (4.4 to 25.5)*	22.7 (5.9 to 42.2)*	0.9 (-5.6 to 7.9)	-8.2 (-17.7 to 2.3)
0–10	9.6 (-1.7 to 22.3)	14.6 (-3.9 to 36.6)	-0.7 (-8.7 to 8.1)	-6.0 (-18.0 to 7.9)

Abbreviation: Pct, percentile

*P<0.05

†Estimates are adjusted for long-term trends, seasonality, humidity, PM_{10}, O_3, day of the week, holidays and influenza epidemics. Minimum apparent temperature was modelled using a natural cubic spline–natural cubic spline DLNM with 2 df for temperature and 3 df for the lag structure in the warm season and with 3 df for temperature and 4 df for the lag structure in the cold season.

Cold season

For minimum as well as maximum temperature, the final model for the cold season contained 3 df for temperature and 4 df for the lag structure. Although unexpected in the cold season, both indicators showed an increase in the risk of preterm birth at higher winter temperatures which is largest at lag 0 (Figures 1C, 1D). On the other hand, the effect of low temperatures appeared to be smaller and was delayed by few days for both temperature indicators. The increase in preterm birth with decreasing temperature was relatively linear for maximum temperature. For minimum temperature, however, the risk of preterm birth was largest around -2°C (approximately the 10th percentile of minimum temperature in the cold season) and decreased again at lower temperatures. The only significant cold effect observed was for minimum temperature at lag 2 (Figure 2C): for a decrease in minimum temperature from the median (4.5°C) to the 5th percentile (-3.3°C), the increase in the risk of preterm birth was 3.3% (95% CI 0.1 to 6.6). Because of the negative estimates at lag 0, however, cumulative cold effects were not significant (Table 3).

Subpopulation analyses

Figure 3 presents the cumulative heat effects and cold effects over lag 0–3 days estimated for different subpopulations. Significant heat effects (Figure 3A), were found for moderate to late preterm births (32-36 weeks) as well as early term (37-38 weeks) and late term (>38 weeks) births (the two latter groups not being part of the main analysis). Although there is a trend of increasing heat effect estimates with decreasing gestational age, significance was lost for the extremely to very preterm births (<32 weeks). Heat effects appeared to be highest for female babies, for mothers with previous births, for older mothers, for mothers with a medium educational level and for urban/semi-urban place of residence, but differences between estimates were not significant. Estimates of the cumulative cold effect (Figure 3B) did not reach significance in any of the subpopulations.

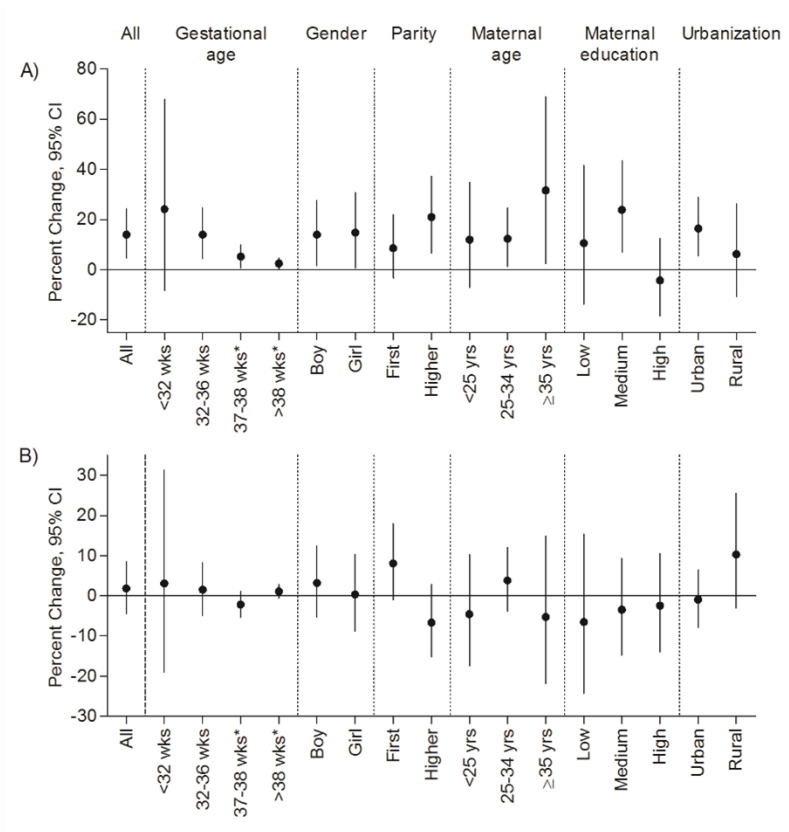

Figure 3. Percent change (with 95% CI) in the risk of preterm birth for an increase in minimum temperature (lag 0–3) from the median (12.8°C) to the 95th percentile (17.9°C) in the warm season (A) and for a decrease in minimum temperature (lag 0–3) from the median (4.5°C) to the 5th percentile (-3.3°C) in the cold season (B), overall and by subpopulations, Flanders, Belgium, May 1998 – April 2011. * Term births (37–38 and >38 weeks) are not included in the main analyses (All).

Sensitivity analyses

The exclusion of air pollutants from the model for the warm season resulted in a slight decrease in estimates (results not shown), which was mainly caused by the exclusion of ozone. For the cold season, results from models with and without air pollutants were practically identical. Changing the df for long-term trends and seasonality gave similar estimates (results not shown). As expected, including lag 0 as a separate term into the model (but excluding it from the cross-basis) affected the estimates for the nearest lags, but conclusions remained the same (supplementary Figure S1).

Discussion

We observed significant effects of recent minimum ambient temperature on the risk of preterm delivery. The heat effect was acute, lasted for a few days, and increased with increasing temperature. The effect of cold, however, was only observed for relatively mild temperatures (between the 50th and the 95th percentile) two days before birth (lag 2). This finding might be explained by the fact that, via clothing and heating, it is easier to adjust to low temperatures than to high temperatures. Whereas virtually all houses in Belgium have heating equipment, only very few houses are equipped with air conditioning.[35] The importance of night-time temperature and a decrease in estimates for the coldest temperatures were also found in a study on hospital admissions in Italy.[36]

A review of the epidemiological literature on the influence of ambient temperature on birth outcomes concluded that findings for preterm delivery are inconclusive.[13] As far as we know, only one previous study has found an association between heat and preterm birth in a colder climate,[17] whereas studies on populations in Montreal, London and Germany did not find an association.[15 16 18] In warmer climates, effects of heat on preterm birth have been reported for Negev, California, Barcelona, Brisbane, Rome and Valencia,[21 22 29 37-40] but not for New York and Chicago.[41 42]

Although the heat effect was not significant for extremely to very preterm births, probably due to the small number of cases, estimates increased with decreasing gestational age. This is in contrast with findings from previous studies, showing smaller or even negative estimates for low gestational ages.[21 22 37] Our finding of a smaller but significant heat effect for term births compared to preterm births, however, is consistent with previous studies.[21 37] Auger *et al.*[16] found an association between high temperatures and the risk of delivery among term pregnancies, with higher estimates for early-term than for full-term births, but not among preterm pregnancies.

Although differences in heat effects between subpopulations were not significant, results suggested a somewhat higher vulnerability in some groups. Girls had a slightly more elevated risk compared with boys, consistent with findings from California.[37] Heat effects seemed more pronounced for older mothers (≥35 years), whereas previous studies reported a tendency of decreasing heat estimates for

increasing maternal age.[22 37] The absence of heat effects among mothers with a higher education is consistent with results for Rome.[22]

Results of this study suggest that night-time temperature might be more relevant than daytime as a trigger for health-related conditions. Minimum temperature reflects the temperature decrease during night which might be important during the warmer period of the year. High temperatures at night means that there is no relief from heat stress and might lead to a worse sleep quality, which has been found to be associated with an increased risk of preterm delivery.[43 44]

The discrepancy between results for minimum and maximum temperature might also be related to the chronology of events, which is less clear for maximum temperature, because at lag 0 the birth precedes the exposure for a considerable number of cases. Minimum temperatures, however, generally occur around or shortly after sunrise, so a much larger proportion of women actually experienced the lag 0 exposure before giving birth. Moreover, the timing of minimum temperatures is far less variable than the timing of maximum temperatures. In summer, maximum temperatures can occur as late as 5 pm, whereas in winter they tend be closer to around 1 pm. This might explain why the differences between results for both temperature indicators seem to be larger in the warm season than in the cold season. However, a sensitivity analysis showed that excluding lag 0 from the cross-basis did not remove the discrepancy between estimates for both temperature indicators.

Different results for minimum and maximum temperature were also reported for the warm season in Valencia:[29] compared with the median temperature, the risk of preterm birth increased up to 20% when maximum apparent temperature exceeded the 90th percentile two days before delivery and 5% when minimum temperature rose to the 90th percentile during the 4th to 6th day before delivery. The authors found an apparently negative association between preterm birth and exposure on the day of birth for maximum apparent temperature, but not for minimum temperature.

Can we explain the current epidemiologic associations by biological mechanisms? Relative extremes of temperature are known to affect human blood flow with excess cardiovascular deaths occurring during heat waves and cold spells.[45-47] It is, therefore, plausible that maternal blood flow and hence fetal nutrition will be

affected by temperature extremes at different stages of gestation. Heat stress can lead to uterine contractions in pregnant women.[48 49] Studies in ewes showed that heat stress might cause an increased secretion of oxytocin and prostaglandin F2α,[49 50] which are involved in the induction of labor. High ambient temperatures are associated with preeclampsia, which is a major cause of preterm birth.[8] Also the release of heat-shock proteins is linked to preterm delivery through induction of proinflammatory cytokines.[51 52] Finally, heat stress may lead to preterm delivery through an increased secretion of corticotrophin-releasing hormone and cortisol.[53 54]

A limitation of our study is the use of ecological-level outdoor measurements for ambient temperature. This could result in exposure misclassification because pregnant women may spend a lot of time indoors, where levels might be different because of the use of air-conditioning and/or heating. As discussed above, this might explain the absence of significant cold effects at very low temperatures. On hot days, however, outdoor temperature might be a better reflection of the true exposure, because, compared with the US for example, the proportion of houses equipped with air conditioning in Belgium is relatively low.[35] Although Flanders is quite uniform for temperature, urban temperatures can be considerably higher than those in the countryside. This probably explains the much larger heat effect observed for mothers living in urban compared to rural areas.

Another limitation is the lack of information on some important risk factors for preterm birth, such as maternal comorbidity, nutrition, drinking and smoking behavior. However, by using a time-series design non-time-varying individual risk factors are inherently controlled for. Moreover, the temporal variation and the gestational age distribution of the pregnancies at risk for preterm birth are properly accounted for. Finally, results are based on a relatively large population and a long-time span.

Conclusion

Our results suggest that pregnant women should protect themselves from temperature extremes. With future climate projections including increases in the frequency and intensity of extreme weather events, and given that even a mild reduction in gestational age has been linked to adverse health outcomes in early and later life, our study may have important implications for public health.

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Supplementary material

Table S1. Percent change (with 95% CI) in the risk of preterm birth associated with high maximum temperatures (95th and 99th percentile) in the warm season and low maximum temperatures (5th and 1st percentile) in the cold season, relative to the median values of the season, Flanders, Belgium, May 1998 – April 2011

	Percent change (95% CI)†			
	WARM season (50th Pct = 20.6°C)		COLD season (50th Pct = 9.8°C)	
Lag (day)	95th Pct = 29.0°C	99th Pct = 31.9°C	5th Pct = 1.1°C	1st Pct = -1.4°C
0	0.1 (-5.4 to 5.9)	-0.5 (-8.9 to 8.6)	-5.1 (-11.0 to 1.2)	-6.9 (-15.1 to 2.0)
0–1	0.7 (-8.1 to 10.3)	-0.2 (-13.5 to 15.1)	-4.0 (-10.3 to 2.7)	-5.8 (-14.5 to 3.8)
0–2	1.6 (-9.0 to 13.3)	0.8 (-15.1 to 19.7)	-1.3 (-7.6 to 5.5)	-2.6 (-11.5 to 7.1)
0–3	2.6 (-8.7 to 15.4)	2.3 (-15.0 to 23.0)	-0.2 (-7.0 to 7.1)	-1.7 (-11.3 to 8.9)
0–5	4.7 (-7.3 to 18.2)	5.3 (-13.1 to 27.7)	-2.7 (-9.4 to 4.5)	-5.9 (-15.2 to 4.5)
0–10	4.2 (-9.3 to 19.7)	4.3 (-16.4 to 30.2)	-1.3 (-9.6 to 7.8)	-6.1 (-17.7 to 7.1)

Abbreviation: Pct, percentile

*P<0.05

†Estimates are adjusted for long-term trends, seasonality, humidity, PM_{10}, O_3, day of the week, holidays and influenza epidemics. Maximum apparent temperature was modelled using a natural cubic spline–natural cubic spline DLNM with 2 df for temperature and 3 df for the lag structure in the warm season and with 3 df for temperature and 4 df for the lag structure in the cold season.

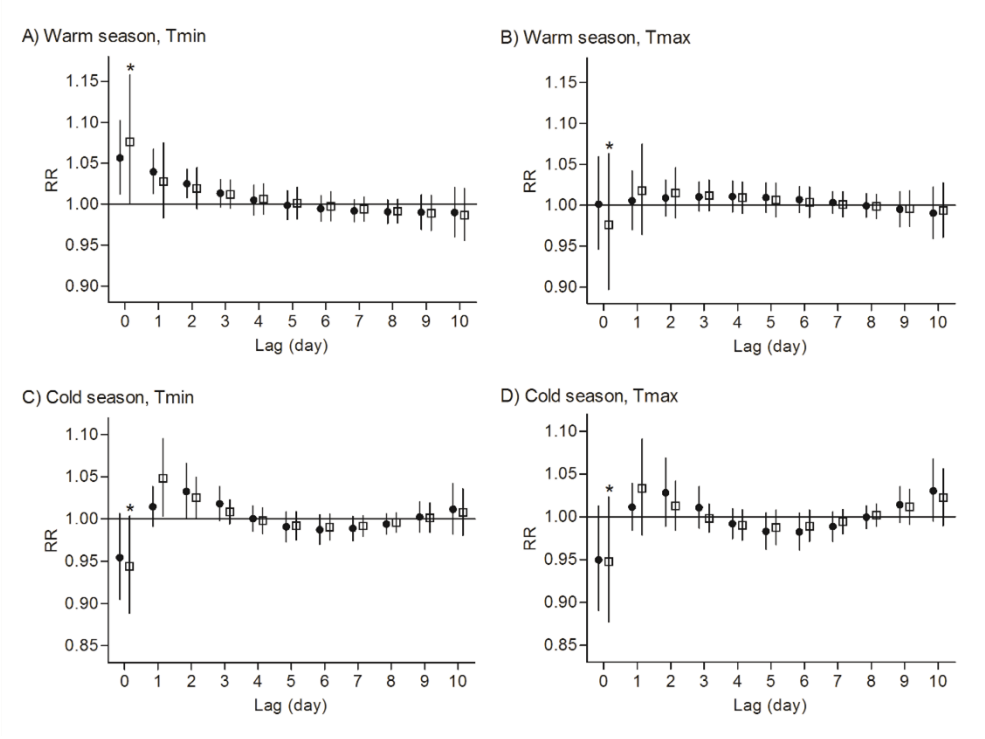

Figure S1. Comparison of lag-specific effects of minimum (Tmin) and maximum (Tmax) temperature on preterm birth between models including (solid circles) and excluding (open squares) lag 0 from the DLNM cross-basis, Flanders, Belgium, May 1998 – April 2011. Heat effects (warm season: A, B) are estimated at the 95th percentile (17.9°C for Tmin and 29.0°C for Tmax), relative to the median (12.8°C for Tmin and 20.6°C for Tmax). Cold effects (cold season: C, D) are estimated at the 5th percentile (-3.3°C for Tmin and 1.1°C for Tmax), relative to the median (4.5°C for Tmin and 9.8°C for Tmax). The symbols are mean relative risks (RR) and the error bars are 95% CIs. *For the models excluding lag 0 from the cross-basis, the effect at lag 0 was estimated by a natural cubic spline with 3 df.

Chapter 5

Fetal growth and maternal exposure to particulate air pollution – More marked effects at lower exposure and modification by gestational duration

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Abstract

While there is growing evidence that air pollution reduces fetal growth, results are inconclusive with respect to the gestational window of effect. We investigated maternal exposure to particulate matter (PM_{10}) in association with birth weight and fetus growth with a focus on the shape of the association and gestational age at birth as a potential effect modifier.

The study population consisted of 525,635 singleton live births in Flanders (Belgium) between 1999 and 2009. PM_{10} exposure at maternal residence was averaged over various time windows. We used robust linear and logistic regression to estimate the effect of PM_{10} on birth weight and small-for-gestational-age (SGA). Segmented regression models were applied for non-linear associations.

Among moderately preterm (32-36 weeks) and term (>36 weeks) births, we found significant lower birth weight for all studied time windows. The estimated reduction in birth weight for a 10 $\mu g/m^3$ increase in average PM_{10} during pregnancy was 39.0 g (95% CI 26.4 to 51.5) for moderately preterm births and 24.0 g (95% CI 20.9 to 27.2) for term births. The corresponding odds ratios for SGA were 1.19 (95% CI 1.07 to 1.32) and 1.09 (95% CI 1.06 to 1.12) respectively. Segmented regression models showed stronger effects of PM_{10} on fetal growth at lower concentrations.

Maternal PM_{10} exposure was significantly associated with a reduction in fetal growth among term and moderately preterm births, with a tendency of stronger effects for the latter and a flattening out of the slope at higher PM_{10} concentrations.

Introduction

The effects of air pollution exposure on the development of the fetus have become an area of increasing focus. Fetal growth is an important indicator of developmental problems and reduced growth is associated with diseases in adulthood including an elevated risk for (1) cardiovascular problems such as coronary heart disease and stroke,[1] (2) hypertension,[2] (3) type 2 diabetes,[3] and (4) mental issues resulting in an increased trend of prescriptions for antipsychotics, antidepressants, and hypnotics/sedatives in young adulthood.[4]

Several studies investigated whether maternal air pollution exposure is associated with adverse birth outcomes, such as low birth weight (LBW, <2,500 g), small-for-gestational-age (SGA), premature birth (<37 weeks of gestation), birth defects, and stillbirth.[5-7] Most reviews concluded that there is growing evidence for an association between prenatal exposure to air pollution and fetal growth.[5-9] Meta-analyses showed substantial heterogeneity between studies that may result from differences in outcome definitions, air pollutants considered, quantification of exposure, exposure windows, study populations and regions, statistical methods, and (inadequate) adjustment for confounders.[10-12] One of the recommendations is that the variation in effects by exposure window should be further explored. The interpretation of air pollution effects on birth weight is further complicated by the fact that they can reflect an influence on length of gestation, on fetal growth, or both. To capture only the latter, most studies have restricted their study population to term births. Moreover, the majority of studies assumed that the effect of air pollution on fetal growth is linear, whereas a few studies have suggested that this may not be the case.[13 14]

Here, we investigated the association between fetal growth and maternal PM_{10} exposure during different time windows of pregnancy, including some critical exposure windows around the start and end of pregnancy. Relatively novel aspects of this study are the investigation of the shape of the association and the assessment of potential effect modification by gestational age. For the latter, we stratified the analysis by gestational age group (<32, 32-36, >36 weeks).

Methods

Data

The Study Centre for Perinatal Epidemiology (SPE) obtained information on all births in Flanders. Flanders is the Dutch speaking Northern part of Belgium with an area of 13,522 km^2 and a population of about six million people. It has 68 maternity units where 99.8% of all births (≥500 g) occur. For each newborn of at least 500 g, an official perinatal form is completed, mostly by the midwife, which contains information on birth weight and gestational age. The form is sent to the SPE, where an error detection program controls all data and feedback is provided.[15] The qualitative assessment of data obtained by the SPE showed that there is less than 5% difference between electronic data and data derived from medical files.[15] Gestational age is counted as the number of weeks starting from the first day of the last menstrual period and is corrected based on the measured crown-rump length from the first ultrasound. Information on national origin of the mother and education are gathered through linkage of the medical birth certificates of the SPE with official birth declarations. Because this linkage is only available from 1999 to 2009, we only considered births during this time period. We restricted our study population to live-born singleton births delivered at 24-44 weeks of gestation. Outcomes of interest in this study were birth weight and SGA. Neonates were classified as SGA when the birth weight was below the 10^{th} percentile of the birth weight for a given gestational age and gender of the newborn in the study population.

Average daily PM_{10} concentrations were obtained from the Belgian Interregional Environment Agency (IRCEL). In the region of Flanders, nineteen monitoring stations, situated on average 25 km apart from each other, have been in use since 1998. Daily levels of air pollution are interpolated by means of a land use regression model (RIO), described by Janssen and colleagues.[16] This provides interpolated air pollution estimates on a 4 x 4 km^2 grid. Since home address information was not available due to privacy laws in Belgium, population-weighted averages were calculated per municipality. Meteorological data consisting of mean daily air temperature and relative humidity, measured at the central and representative station of Uccle (Brussels, Belgium), were provided by the Belgian Royal Meteorological Institute. Apparent temperature, an index of human

discomfort incorporating relative humidity, was computed by using a standard formula.[17 18]

Maternal PM_{10} exposure and mean apparent temperature were averaged over different time windows: the entire pregnancy, the two weeks around conception (one week before until six days after conception), the two weeks after conception, each of the three trimesters, and the last month of pregnancy. Average exposure during the third trimester was only calculated for moderately preterm and term births, because this time window is very short or non-existent for extremely preterm births (<32 weeks). The research protocol was approved by the medical ethics committee of the Hasselt University.

Statistical analyses

To study the association between fetal growth and PM_{10}, we applied robust linear regression models for the continuous outcome birth weight and logistic models for the binary outcome SGA. Since we expected the effects of PM_{10} exposure and covariates on birth outcomes to depend on gestational age, analyses were performed within the following three groups: extremely preterm (<32 weeks), moderately preterm (32-36 weeks), and term (>36 weeks) births.

The shape of the association between birth outcomes and maternal PM_{10} exposure was explored by the use of smoothing plots (natural cubic splines with 4 degrees of freedom). Because different exposure windows showed a breakpoint in the dose-response curve with relatively linear effects before and after that point, we additionally performed segmented (piecewise linear) regression analyses.

The choice of covariates adjusted for in the analysis was based on previous study findings on this topic.[19-21] Models included indicator variables for year of birth, season of conception (winter, spring, summer, autumn), parity (first, second, higher-order birth), maternal age group (<25, 25-34, >34 years), marital status (married, unmarried), maternal and paternal education (lower secondary or less, higher secondary, higher education), national origin of the mother (Europe, Asia, Middle-East, Africa, North-America, South-America, Oceania), province of residence (West Flanders, East Flanders, Antwerp, Flemish Brabant, Limburg), and a linear term for apparent temperature. For the regression models fitting birth

weight, we additionally adjusted for infant's sex and gestational age (linear and quadratic term).

Population attributable fractions (PAFs) were calculated as the proportion of SGA births that could be avoided if average maternal PM_{10} exposure during pregnancy was below 20 µg/m^3, which is the WHO annual guideline value.[22] PAFs are calculated with the formula $PAF = \sum (OR_{c-20} - 1)/OR_{c-20}$.[23 24] OR_{c-20} is the odds ratio of SGA comparing a maternal exposure concentration *c* with an exposure of 20 µg/m^3.

In a secondary analysis, we examined whether specific subgroups were more vulnerable to the effects of maternal PM_{10} exposure. We stratified by gender of the newborn, parity, maternal age group, maternal and paternal educational level, and season of conception. Because of the small number of extremely and moderately preterm births within subpopulations, these secondary analyses were only performed in the term group.

A total of 122,936 births (19%) was not included in the final study population because of missing values for at least one of the covariates in the model (2,399 missing values for marital status, 41,847 for maternal education, 69,492 for paternal education and 55,492 for maternal origin). In a sensitivity analyses, models without adjustment for aforementioned variables (one model for the total population and one for the final study population) were used to examine the impact of the exclusion of these births.

Estimates are reported for a 10 µg/m^3 increment in PM_{10} exposure. All analyses were performed by using SAS version 9.2 (SAS Institute, Cary, North Carolina, USA). A P-value less than 0.05 was considered significant.

Results

There were 672,261 live births in Flanders between 1999 and 2009. From these, 23,551 (3.5%) non-singleton births and a further 139 (0.02%) births with a gestational age below 24 or above 44 weeks were excluded. After excluding 122,936 (19.0%) deliveries with missing information on one of the covariates, the final study population consisted of 525,635 births. Mean birth weight was 3,350 g and a total of 49,605 (9.4%) of newborns were SGA (Table 1). Of the 30,982

(5.9%) preterm births, 27,912 (90.1%) were moderately preterm and 3,070 (9.9%) were extremely preterm. Most neonates were firstborn (46.6%) from mothers between 25 and 34 years old (72.7%). Mean birth weight was lowest (and the percentage of SGA babies was highest) for girls, firstborns, young mothers, unmarried mothers, and mothers and fathers with low education.

Table 1. Descriptive statistics of the study population (n=525,635), Flanders, 1999-2009. Values are percentages or means (10-90th percentiles).

Characteristic	Value	% of all births	Mean birth weight [g] (10-90th percentiles)	% SGA
Total		100.0	3,350 (2,750-3,960)	9.4
Gestational age	<32 weeks	0.6	1,259 (745-1,795)	9.6
	32-36 weeks	5.3	2,592 (1,940-3,200)	9.5
	>36 weeks	94.1	3,399 (2,850-3,980)	9.4
Gender	Boy	51.3	3,413 (2,800-4,030)	9.4
	Girl	48.7	3,284 (2,700-3,880)	9.5
Parity	1	46.6	3,279 (2,680-3,885)	11.9
	2	35.4	3,408 (2,825-4,000)	7.2
	≥ 3	18.0	3,419 (2,795-4,060)	7.3
Maternal age	<25 years	15.3	3,260 (2,660-3,865)	12.5
	25-34 years	72.7	3,366 (2,770-3,970)	8.8
	>34 years	12.1	3,371 (2,725-4,020)	9.5
Marital status	Unmarried	31.1	3,296 (2,680-3,915)	11.5
	Married	68.9	3,375 (2,780-3,980)	8.5
Maternal education	Low	12.6	3,282 (2,650-3,925)	12.6
	Medium	40.7	3,310 (2,700-3,930)	10.7
	High	46.7	3,405 (2,820-4,000)	7.4
Paternal education	Low	14.7	3,286 (2,660-3,920)	12.4
	Medium	46.9	3,322 (2,710-3,940)	10.3
	High	38.3	3,409 (2,830-4,000)	7.3
Maternal origin	Europe	89.9	3,349 (2,750-3,960)	9.4
	Asia	1.1	3,274 (2,680-3,890)	11.9
	Middle East	3.6	3,321 (2,745-3,920)	10.5
	Africa	4.9	3,410 (2,810-4,038)	8.5
	North-America	0.1	3,449 (2,900-4,050)	5.0
	South-America	0.4	3,337 (2,785-3,916)	8.8
	Oceania	0.0	3,304 (2,710-3,880)	10.2
Season of conception	Winter	23.5	3,346 (2,740-3,960)	9.6
	Spring	25.0	3,344 (2,740-3,950)	9.7
	Summer	25.4	3,356 (2,760-3,970)	9.2
	Autumn	26.2	3,354 (2,750-3,970)	9.3

Abbreviation: SGA, Small-for-gestational-age

Table 2 provides the distribution of average PM_{10} exposures during the different time windows. Average PM_{10} was close to 31 µg/m^3 for all windows. Trimester exposures were highly correlated with entire pregnancy exposure (linear correlation coefficient (r) >0.8) and moderately correlated with each other (r between 0.5 and 0.7) (Table 3). Correlations between time windows around conception and late pregnancy periods were low (r<0.4).

Table 2. Distribution of PM_{10} [µg/m^3] exposure in different time windows during pregnancy, Flanders, 1999-2009

			Percentiles			
Exposure window	**Mean**	**(SD)**	**5th**	**25th**	**75th**	**95th**
Entire pregnancy	31.24	(-5.88)	22.42	26.76	35.53	41.46
Two wks around conception	31.52	(-9.60)	18.33	24.84	36.71	49.37
Two wks after conception	31.55	(-9.62)	18.34	24.87	36.74	49.40
First trimester	31.46	(-6.54)	21.56	26.73	35.84	42.78
Second trimester	31.25	(-6.70)	21.03	26.42	35.73	42.84
Third trimester	30.96	(-7.07)	20.09	25.86	35.60	43.32
Last month	30.87	(-8.18)	18.73	25.00	35.92	45.51

Abbreviation: SD, Standard deviation

Table 3. Correlation coefficients between average PM_{10} exposure in different time windows during pregnancy, Flanders, 1999-2009*

Exposure window	Entire pregnancy	Two wks around conception	Two wks after conception	First trimester	Second trimester	Third trimester	Last month
Entire pregnancy	1						
Two wks around conception	0.53	1					
Two wks after conception	0.56	0.75	1				
First trimester	0.86	0.58	0.65	1			
Second trimester	0.90	0.42	0.42	0.67	1		
Third trimester	0.84	0.39	0.38	0.56	0.67	1	
Last month	0.72	0.35	0.34	0.49	0.55	0.86	1

*P<0.001 for all correlations

Among moderately preterm and term births, we observed significant negative associations between PM_{10} and birth weight for all studied time windows, whereas we did not find any significant association among extremely preterm births (Table 4). Effects of PM_{10} on birth weight were always stronger for moderately preterm than for term births and were highest for entire pregnancy exposure in both groups: for a 10 µg/m^3 increase in average PM_{10} during pregnancy, birth weight

decreased by 39.0 g (95% CI 26.4 to 51.5) among moderately preterm births and by 24.0 g (95% CI 20.9 to 27.2) among term births.

Smoothing plots of the association between PM_{10} and change in birth weight suggested the existence of a breakpoint in the shape of the association, with relatively linear slopes before and after the breakpoint. Slopes were steepest at lower levels of PM_{10} (below approximately 35 $\mu g/m^3$) and flattened out at higher levels. The shape of the association is shown for entire pregnancy (Figure 1A) and first trimester (Figure 1B) PM_{10} exposure among term births. Supplementary Figures S1 and S2 display the exposure-response curves for the other pregnancy windows and gestational age groups. The decrease in slope above the breakpoint was significant for all time windows among term births, and for the time windows two weeks around conception and second trimester among moderately preterm births. Estimates for the significant breakpoints varied from 29.8 to 40.5 $\mu g/m^3$.

Table 4. Association between birth weight and maternal exposure to PM_{10} in different time windows during pregnancy

	Linear regression	Segmented linear regression			
	Change in weight [g]	Breakpoint [µg/m³]	P-value†	Change in weight [g] below breakpoint	Change in weight [g] above breakpoint
>36 weeks (n=494,653)					
Entire pregnancy	-24.0 (-27.2 to -20.9)*	35.4 (31.9 to 38.9)	<0.0001	-28.3 (-32.3 to -24.3)*	-14.2 (-20.7 to -7.6)*
Two wks around conception	-4.0 (-5.5 to -2.8)*	29.8 (24.4 to 35.3)	<0.0001	-10.9 (-13.0 to -6.0)*	-1.9 (-3.8 to -0.1)*
Two wks after conception	-5.0 (-7.0 to -3.6)*	33.9 (28.6 to 39.2)	<0.0001	-8.7 (-11.4 to -6.1)*	-2.0 (-4.2 to 0.2)
First trimester	-14.8 (-17.4 to -12.3)*	40.5 (37.9 to 43.1)	<0.0001	-17.4 (-20.3 to -14.5)*	5.7 (-3.7 to 15.0)
Second trimester	-16.6 (-19.1 to -14.0)*	31.2 (23.4 to 38.9)	0.041	-20.6 (-25.0 to -16.1)*	-14.2 (-17.7 to -10.7)*
Third trimester	-14.8 (-17.2 to -12.4)*	36.2 (31.7 to 40.7)	<0.0001	-18.1 (-21.3 to -15.0)*	-6.8 (-11.8 to -1.7)*
Last month	-8.8 (-10.6 to -7.0)*	31.9 (24.1 to 39.7)	0.013	-12.1 (-15.5 to -8.8)*	-5.9 (-8.7 to -3.2)*
32-36 weeks (n=27,912)					
Entire pregnancy	-39.0 (-51.5 to -26.4)*	32.6 (24.1 to 41.1)	0.071	N/A	N/A
Two wks around conception	-13.9 (-19.5 to -8.2)*	31.5 (23.0 to 40.0)	0.007	-29.1 (-41.5 to -16.6)*	-5.4 (-13.6 to 2.8)
Two wks after conception	-11.7 (-17.3 to -6.1)*	29.4 (18.7 to 40.1)	0.523	N/A	N/A
First trimester	-29.0 (-39.4 to -18.6)*	29.9 (15.7 to 44.0)	0.766	N/A	N/A
Second trimester	-26.0 (-36.5 to -15.5)*	33.7 (26.9 to 40.4)	0.033	-38.0 (-53.3 to -22.6)*	-10.5 (-27.9 to 7.0)
Third trimester	-16.5 (-25.0 to -7.9)*	30.6 (2.2 to 59.0)	0.690	N/A	N/A
Last month	-10.2 (-17.7 to -2.7)*	14.6 (11.4 to 17.8)	0.344	N/A	N/A
<32 weeks (n=3,070)					
Entire pregnancy	6.5 (-18.8 to 31.8)	30.6 (25.5 to 35.8)	0.110	N/A	N/A
Two wks around conception	0.5 (-12.6 to 12.4)	44.6 (14.0 to 75.1)	0.804	N/A	N/A
Two wks after conception	-5.5 (-17.4 to 6.5)	30.6 (20.7 to 40.5)	0.151	N/A	N/A
First trimester	9.6 (-11.6 to 30.8)	26.6 (22.9 to 30.3)	0.060	N/A	N/A
Second trimester	-1.8 (-23.9 to 20.1)	43.1 (24.7 to 61.4)	0.620	N/A	N/A
Last month	5.1 (-10.2 to 20.4)	30.3 (23.6 to 37.0)	0.078	N/A	N/A

Estimates (95% CI) are expressed as the change in birth weight for a 10 µg/m³ increase in PM_{10} and are adjusted for year of birth, season of conception, parity, maternal age group, marital status, maternal and paternal education, national origin of the mother, province of residence, apparent temperature, infant's sex and gestational age.

*P<0.05

†Significance of the change in slope above the breakpoint; N/A = Not applicable (slope change not significant)

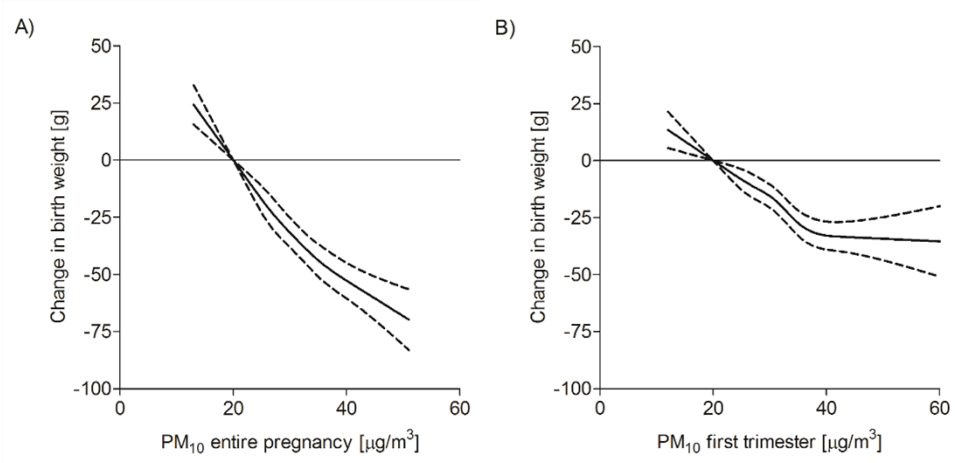

Figure 1. Shape of the association between term birth weight and maternal PM_{10} exposure during the entire pregnancy (A) and during the first trimester (B). Estimates (solid line) and 95% CI (dashed lines) represent the change in birth weight relative to the reference value of 20 µg/m^3. PM_{10} exposure was modeled using a natural cubic spline with 4 degrees of freedom and estimates are adjusted for year of birth, season of conception, parity, maternal age group, marital status, maternal and paternal education, national origin of the mother, province of residence, apparent temperature, infant's sex, and gestational age.

Similar results were found when considering SGA as an outcome (Table 5). For moderately preterm and term births, the association between SGA and maternal PM_{10} exposure was mostly significant (except for some time windows at the end of pregnancy), but no significant associations were found among extremely preterm births. Odds ratios for a 10 µg/m^3 increase in PM_{10} were highest for the entire pregnancy time window: 1.19 (95% CI 1.07 to 1.32) for moderately preterm births and 1.09 (95% CI 1.06 to 1.12) for term newborns. The population attributable fraction for a decrease in average PM_{10} exposure during pregnancy to 20 µg/m^3 was 24% (95% CI 10 to 35) for moderately preterm SGA newborns and 13% (95% CI 9 to 16) for term SGA newborns. The decrease in slope after the breakpoint was significant for the time windows entire pregnancy, two weeks after conception, and second trimester among term births, and the time windows entire pregnancy and last month among moderately preterm births. Estimates for the significant breakpoints ranged from 27.2 to 40.0 µg/m^3.

Table 5. Association between SGA and maternal exposure to PM_{10} in different time windows during pregnancy

	Logistic regression	Segmented logistic regression			
	Odds ratio SGA	Breakpoint [µg/m³]	P-value†	Odds ratio SGA below breakpoint	Odds ratio SGA above breakpoint
>36 weeks (n=494,653)					
Entire pregnancy	1.09 (1.06 to 1.12)*	27.2 (23.5 to 31.0)	0.017	1.19 (1.10 to 1.28)*	1.07 (1.04 to 1.11)*
Two wks around conception	1.01 (1.00 to 1.02)*	30.0 (26.8 to 33.3)	0.098	N/A	N/A
Two wks after conception	1.02 (1.01 to 1.03)*	31.5 (27.9 to 35.1)	0.049	1.04 (1.02 to 1.07)*	1.01 (0.99 to 1.02)
First trimester	1.06 (1.04 to 1.08)*	35.5 (24.4 to 46.5)	0.701	N/A	N/A
Second trimester	1.07 (1.05 to 1.10)*	29.1 (24.8 to 33.3)	0.007	1.13 (1.08 to 1.19)*	1.05 (1.02 to 1.08)*
Third trimester	1.04 (1.02 to 1.06)*	26.9 (22.0 to 31.8)	0.193	N/A	N/A
Last month	1.02 (1.00 to 1.03)*	31.5 (25.5 to 37.5)	0.382	N/A	N/A
32-36 weeks (n=27,912)					
Entire pregnancy	1.19 (1.07 to 1.32)*	34.5 (24.5 to 44.5)	0.028	1.33 (1.14 to 1.53)*	0.98 (0.79 to 1.20)
Two wks around conception	1.09 (1.04 to 1.15)*	32.2 (16.2 to 48.2)	0.447	N/A	N/A
Two wks after conception	1.07 (1.02 to 1.12)*	14.3 (11.4 to 17.1)	0.453	N/A	N/A
First trimester	1.16 (1.06 to 1.27)*	54.4 (48.1 to 60.7)	0.248	N/A	N/A
Second trimester	1.13 (1.03 to 1.24)*	51.5 (37.5 to 65.6)	0.343	N/A	N/A
Third trimester	1.04 (0.97 to 1.12)	17.8 (8.4 to 27.2)	0.215	N/A	N/A
Last month	1.01 (0.94 to 1.08)	40.0 (32.1 to 47.9)	0.004	1.09 (1.00 to 1.18)*	0.77 (0.64 to 0.94)*
<32 weeks (n=3,070)					
Entire pregnancy	0.96 (0.70 to 1.34)	23.3 (17.4 to 29.2)	0.054	N/A	N/A
Two wks around conception	1.04 (0.89 to 1.21)	14.9 (14.0 to 15.7)	0.665	N/A	N/A
Two wks after conception	1.12 (0.97 to 1.31)	68.2 (26.7 to 109.6)	0.697	N/A	N/A
First trimester	0.98 (0.74 to 1.29)	26.1 (20.3 to 31.8)	0.069	N/A	N/A
Second trimester	0.94 (0.70 to 1.24)	17.0 (16.6 to 17.3)	0.907	N/A	N/A
Last month	0.96 (0.79 to 1.17)	31.7 (19.6 to 43.9)	0.101	N/A	N/A

Estimates (95% CI) are expressed as odds ratios for the risk of small-for-gestational-age (SGA) for a 10 µg/m³ increase in PM_{10} and are adjusted for year of birth, season of conception, parity, maternal age group, marital status, maternal and paternal education, national origin of the mother, province of residence and apparent temperature.

*P<0.05

†Significance of the change in slope above the breakpoint; N/A = Not applicable (slope change not significant)

Secondary analyses indicated that the association between birth weight and maternal exposure to air pollution was stronger for increasing number of previous births and weaker for increasing maternal age and parental education (Figure 2A). The higher susceptibility of younger mothers was also observed for SGA (Figure 2B).

Models without adjustment for marital status, maternal and paternal education, and national origin of the mother (supplementary Table S1) suggested that estimates were fairly robust to the exclusion of subjects with missing covariate information. In these models estimates of weight decrease were slightly lower for the final study population than for the total study population among term births, and vice versa among moderately preterm births. A comparison with results from the main analysis showed that estimates of weight decrease were generally lowest in the main analyses, except for some exposure windows among moderately preterm births.

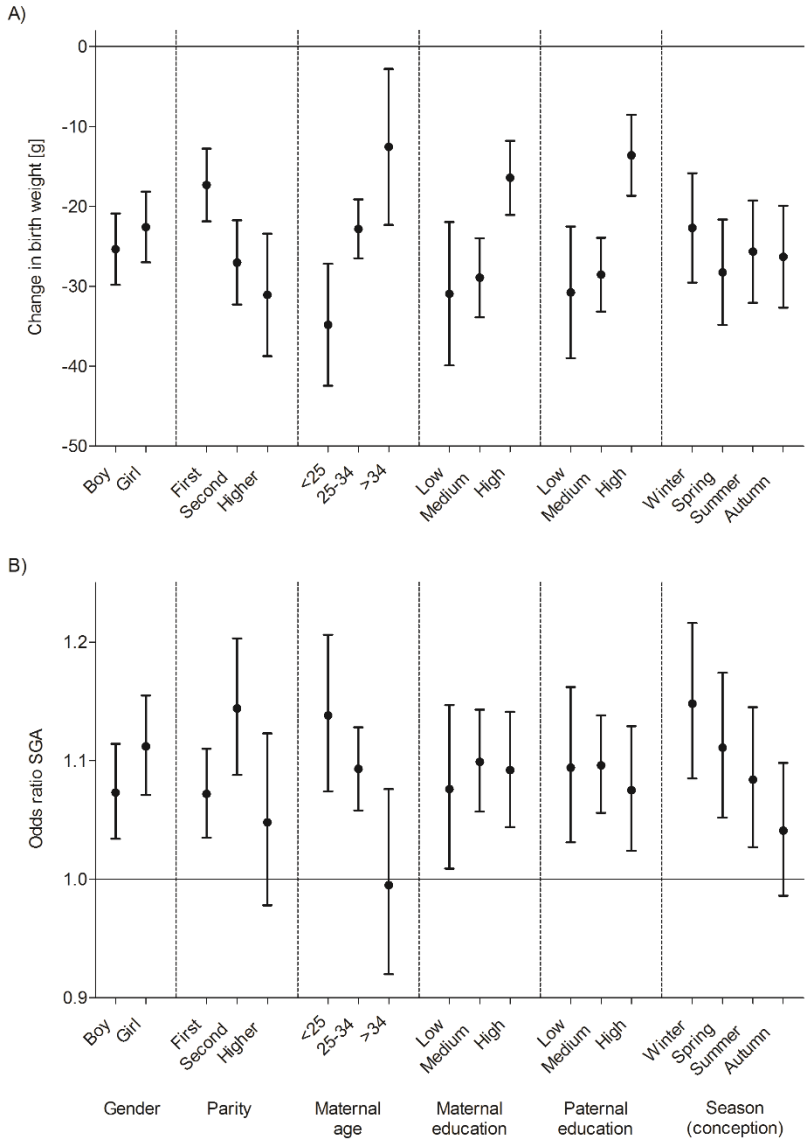

Figure 2. Association of term birth weight (A) and small-for-gestational-age (SGA) (B) with average PM_{10} exposure during pregnancy for different subpopulations. Estimates (point) and 95% CI (error bars) represent the changes in birth weight (A) and odds ratios for SGA (B) for a 10 $\mu g/m^3$ increase in maternal PM_{10} exposure and were adjusted for year of birth, season of conception, parity, maternal age group, marital status, maternal and paternal education, national origin of the mother, province of residence, and apparent temperature and for birth weight (A) additionally for infant's sex, and gestational age.

Discussion

For infants born after 31 weeks of gestation, we observed significant associations between *in utero* PM_{10} exposure and birth weight as well as SGA. The effects of maternal PM_{10} exposure on both outcomes were considerably higher for moderately preterm neonates (32-36 weeks) than for those born at term. Susceptibility to air pollution was found to be higher for multiparous women and for lower maternal age and parental education. For both birth weight and SGA, the estimated breakpoint and the significance of the change in slope depended on the studied time window. In general, significant breakpoints were estimated to lie around 35 $\mu g/m^3$ and the estimated decrease in birth weight was largest for PM_{10} concentrations below this level. Twenty seven percent of our study population had an average pregnancy exposure above 35 $\mu g/m^3$.

Effect modification by gestational age was also observed in the association between second-trimester traffic-related air pollution (nitrogen dioxide, NO_2) and fetal growth restriction with a stronger effect among women subsequently delivering preterm.[25] However, these authors did not examine potential differences in susceptibility between early and late preterm babies. We did not observe a significant association between air pollution exposure and fetal growth for extremely preterm births. Since most of the fetal growth occurs in the third trimester, it might be that extremely preterm babies were born too early to see the effect of air pollution on growth. On the other hand, term babies had a longer time *in utero* to compensate for the effects of exposure, which might explain why smaller effects were observed in term births than in moderately preterm births.

Our estimate for the decrease in birth weight associated with entire pregnancy PM_{10} exposure (for an increase of 10 $\mu g/m^3$: 24.0 g, 95% CI 20.9 to 27.2) is considerably higher than estimates from previous studies. A meta-analysis including 7 studies showed a significant decrease in birth weight of 16.8 g (95% CI 13.3 to 20.2) as entire pregnancy PM_{10} exposure increased with 20 $\mu g/m^3$.[12] Dadvand *et al.*[10] combined effect estimates of 14 study centers established all over the world and observed a significant decrease in birth weight of 8.9 g (95% CI 4.6 to 13.2) for each 10 $\mu g/m^3$ increase in entire pregnancy exposure to PM_{10}. However, a pooled analysis of data from 14 population-based mother-child cohorts

in 12 European countries showed no significant association between PM_{10} exposure and birth weight (for an increase of 10 μg/m^3: -8 g, 95% CI -19 to 3).[26]

For SGA our results are consistent with a recent meta-analysis which found a positive association between SGA and entire pregnancy exposure to particulate matter with diameter less than 2.5 μg/m^3 ($PM_{2.5}$) (OR for an increase of 10 μg/m^3: 1.15, 95% CI 1.10 to 1.20).[27] For $PM_{10,}$ results of previous studies are inconsistent. One study reported a significant association between SGA and PM_{10} in the first gestational month (OR for an increase of 10 μg/m^3: 1.19, 95% CI 1.06 to 1.33) in Teplice.[28] Another study showed an association between SGA and exposure to PM_{10} in the second trimester, with an OR of 1.01 (95% CI 1.00 to 1.04) for a 1 μg/m^3 increase.[29] Hannam and colleagues[30] found a significant association between SGA and entire pregnancy PM_{10} exposure (OR for an increase from the 1st to the 4th quartile: 1.14, 95% CI 1.01 to 1.29). Other studies[31 32] did not find significant associations between PM_{10} exposure and the risk for SGA.

The variability among study results may be due to differences in confounder adjustments, exposure assessment, study populations, and PM_{10} composition. Since the chemical composition of PM_{10} depends on the environmental air pollution sources, it may vary both spatially and temporally. The variety of sources causing the formation of PM_{10} may also emit other air pollutants such as sulphur dioxide (SO_2) and NO_2, which may have contributed to the observed effects. Also, as we found effect modification by variables such as parity, maternal age, parental education, and gestational duration, a different distribution of these or other variables between the studied populations could play a role in the observed differences in effect estimates.

We did not only consider exposure during pregnancy but also periconceptional exposure because air pollution may affect sperm cells and ova through genetic or epigenetic mechanisms,[33] and because placental methylation status at birth has been found to depend on exposures around implantation.[34] These early life exposure windows were significantly associated with both outcomes. The high correlation between entire pregnancy and trimester exposures hampers the identification of the time window in which the fetus is most vulnerable to air pollution. However, the observed effects tend to be higher for exposure in the first trimester than for exposure late in pregnancy (third trimester and last month),

especially for SGA and for moderately preterm births. The stronger association observed for entire pregnancy exposure compared with other time windows might indicate the importance of chronic exposure. However, because longer time windows typically have a smaller range and lower variability in exposure values than shorter windows, a 10 μg/m^3 increase in average PM_{10} for the entire pregnancy is relatively larger than the same increase for a shorter exposure window.

Some other studies investigated the shape of the association between adverse birth outcomes and ambient air pollution. Some conducted a linear analysis after an initial exploration of the shape.[25 35] Ha *et al.*[14] found a significant association between birth weight and air pollution (SO_2, NO_2, total suspended particles and carbon monoxide) exposure in the first trimester. They allowed for nonlinear associations but concluded that the relations were relatively linear, without thresholds for concentrations of the pollutants. Similar to our study, Ballester *et al.*[13] showed a nonlinear association between birth weight and NO_2 exposure. However, we found a flattening out of the slope of the association between birth weight and PM_{10} at higher concentrations (above 30-40 μg/m^3), whereas they showed an increase in slope at higher NO_2 concentrations. A decrease in the effect of exposure at higher exposure levels was also observed for outcomes such as cardiovascular mortality,[36] lung and bladder cancer,[37] and respiratory epithelium integrity.[38] Weaker cardiovascular effects at higher particulate matter exposure may be caused by the saturation of underlying biochemical and cellular processes with small doses of harmful components.[39] Such saturation might also be the cause of the non-linear association in the current study.

The long study period and the large study population of nearly 530,000 newborns, including 30,982 preterm births, is a major strength of our study and enabled the investigation of effect modification by gestational age and many other factors, while maintaining sufficient statistical power. We observed effect modification by a number of factors including parity, maternal age, and parental education. The higher susceptibility observed for parents with lower education levels is consistent with previous observations.[40] Further, we observed a stronger effect for babies from young mothers (<25 years) compared to older mothers. Finally, we found higher effect estimates for higher parity newborns (≥2) than for firstborns. This is compatible with the hypothesis of Ritz and Yu[41] that exposure misclassification

is lower for parous mothers who tend to stay more at home to care for their other children.

Although we controlled for a number of potential confounders, we did not have information on some known important risk factors for birth weight and SGA. Birth certificates did not provide any indication of maternal nutrition, drinking and smoking behavior. On the other hand, a large number of important covariates was taken into account: year of birth, season of conception, parity, maternal age group, marital status, maternal and paternal education, national origin of the mother, province of residence, apparent temperature, infant's sex, and gestational age. It is reasonable to assume that social economic indicators partly account for nutrition and lifestyle.[40]

Another potential source of bias in our analyses is exposure misclassification. We used interpolated ambient PM_{10} estimates at the level of the municipality (average size of 43.9 km²) as a proxy for individual exposure since home addresses were not available. However, this has several drawbacks. First, air pollution levels vary locally such that pregnant women living close to a major roadway or other pollution sources might be more heavily exposed than women living further away. Secondly, we only considered outdoor PM_{10} exposure, although indoor PM_{10} levels might add considerably to the overall burden of exposure for some mothers. A relevant indoor air pollution sources is environmental tobacco smoke.[42-44] Exposure misclassification might also be caused by pregnant women spending large amounts of time outside their municipality of residence.[45] Further, maternal residence is registered at the time of birth and women might have moved to another region during pregnancy. Therefore, misclassification is expected to be smallest later in pregnancy.[11] The date of conception in our study was estimated based on last menstruation and ultrasounds. An incorrect date of conception is more likely to affect exposure averaged over smaller time windows, such as the two weeks around and after conception. Finally, the exclusion of newborns with missing data could bias the results. Nevertheless, sensitivity analyses showed that our estimates were not altered by exclusion of subjects with missing data.

We expect that potential errors in PM_{10} exposure estimates are more or less the same for different levels of PM_{10} (non-differential misclassification), resulting in an underestimation of effect estimates. In a study of Ostro and colleagues[46]

adjustment of exposure estimates for time-activity pattern information, such as time spent outdoors, led to a 43% increase in the estimated effect of air pollution, suggesting non-differential misclassification if exposure estimates were based on fixed-site monitoring stations. Moreover, another study[47] found stronger effects of air pollution (CO, PM_{10}, and $PM_{2.5}$) exposure if they limited their analysis to women living within 1 mile of a monitoring station. This indicates that our estimated effects of prenatal PM_{10} exposure are likely to be underestimated.

Conclusion

Our study findings indicate that, at PM_{10} levels below current air quality standards, prenatal exposure to particulate air pollution reduces birth weight and increases the risk of babies being small-for-gestational-age, not only among infants born at term, but even more strongly among babies born between 32 and 36 weeks of pregnancy. Assuming causality, 24% of moderately preterm SGA newborns and 13% of term SGA newborns could be prevented if average PM_{10} exposure during pregnancy was decreased to 20 $\mu g/m^3$.

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Supplementary material

Table S1. Association between birth weight and maternal exposure to PM_{10} in different time windows during pregnancy, comparison of main analysis and sensitivity analyses without adjustment for covariates with missing values

	Change in birth weight [g]					
			Sensitivity analyses			
	Main analysis†‡		Final study population‡		Total study population§	
>36 weeks						
Entire pregnancy	-24.0	(-27.2 to -20.9)*	-27.8	(-30.9 to -24.7)*	-29.6	(-32.4 to -26.9)*
Two wks around conception	-4.0	(-5.5 to -2.8)*	-4.9	(-6.3 to -3.5)*	-5.7	(-7.0 to -4.5)*
Two wks after conception	-5.0	(-7.0 to -3.6)*	-5.7	(-7.1 to -4.3)*	-6.6	(-7.9 to -5.3)*
First trimester	-14.8	(-17.4 to -12.3)*	-17.7	(-20.2 to -15.1)*	-19.8	(-22.1 to -17.6)*
Second trimester	-16.6	(-19.1 to -14.0)*	-18.4	(-20.9 to -15.9)*	-19.2	(-21.4 to -16.9)*
Third trimester	-14.8	(-17.2 to -12.4)*	-16.5	(-18.9 to -14.0)*	-17.7	(-19.9 to -15.5)*
Last month	-8.8	(-10.6 to -7.0)*	-9.9	(-11.7 to -8.0)*	-10.9	(-12.5 to -9.2)*
32-36 weeks						
Entire pregnancy	-39.0	(-51.5 to -26.4)*	-40.3	(-52.8 to -27.8)*	-38.7	(-49.7 to -27.7)*
Two wks around conception	-13.9	(-19.5 to -8.2)*	-13.4	(-19.1 to -7.8)*	-11.4	(-16.4 to -6.3)*
Two wks after conception	-11.7	(-17.3 to -6.1)*	-11.7	(-17.3 to -6.1)*	-11.3	(-16.9 to -5.7)*
First trimester	-29.0	(-39.4 to -18.6)*	-28.9	(-39.3 to -18.5)*	-25.4	(-34.6 to -16.2)*
Second trimester	-26.0	(-36.5 to -15.5)*	-27.2	(-37.7 to -16.8)*	-30.5	(-39.7 to -21.2)*
Third trimester	-16.5	(-25.0 to -7.9)*	-16.8	(-25.3 to -8.2)*	-14.9	(-22.5 to -7.3)*
Last month	-10.2	(-17.7 to -2.7)*	-11.0	(-18.5 to -3.6)*	-10.8	(-17.5 to -4.1)*

Estimates (95% CI) are expressed as the change in birth weight for a 10 $\mu g/m^3$ increase in PM_{10} and are adjusted for year of birth, season of conception, parity, maternal age group, province of residence, apparent temperature, infant's sex and gestational age.

*P<0.05

†Additionally adjusted for marital status, maternal and paternal education, national origin of the mother

‡494,653 term births (>36 weeks) and 27,912 moderately preterm births (32-36 weeks)

§609,605 term births (>36 weeks) and 34,958 moderately preterm births (32-36 weeks)

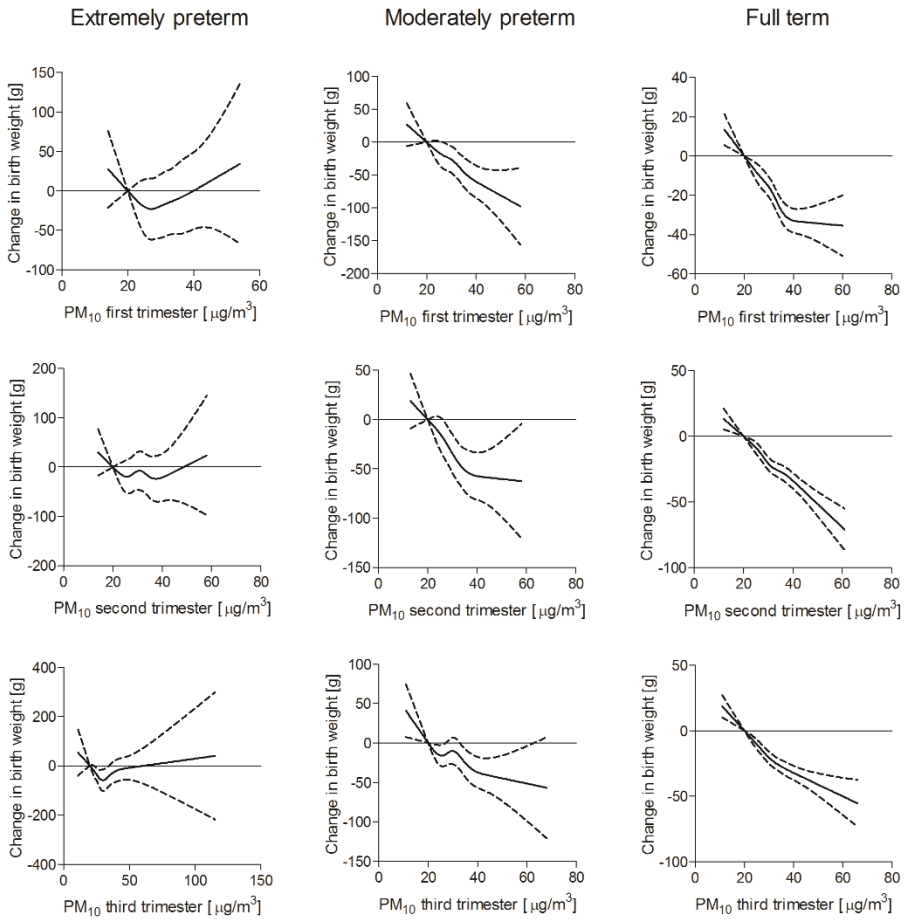

Figure S1. Shape of the association between birth weight and maternal PM_{10} exposure during each of the three trimesters of pregnancy, by gestational age group. Estimates (solid line) and 95% CI (dashed lines) represent the change in birth weight relative to the reference value of 20 μg/m³. PM_{10} exposure was modeled using a natural cubic spline with 4 degrees of freedom and estimates are adjusted for year of birth, season of conception, parity, maternal age group, marital status, maternal and paternal education, national origin of the mother, province of residence, apparent temperature, infant's sex, and gestational age.

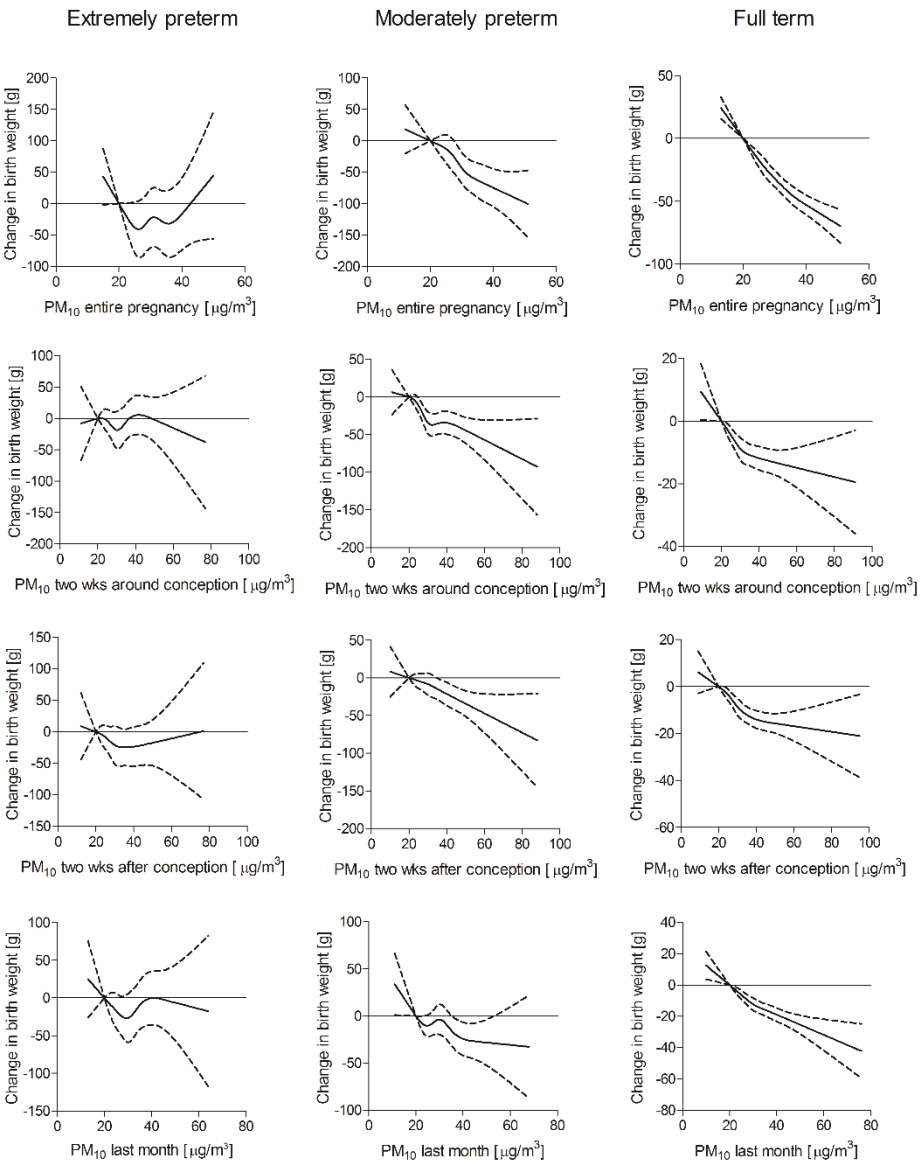

Figure S2. Shape of the association between birth weight and maternal PM_{10} exposure during entire pregnancy and during early and late pregnancy periods, by gestational age group. Estimates (solid line) and 95% CI (dashed lines) represent the change in birth weight relative to the reference value of 20 $\mu g/m^3$. PM_{10} exposure was modeled using a natural cubic spline with 4 degrees of freedom and estimates are adjusted for year of birth, season of conception, parity, maternal age group, marital status, maternal and paternal education, national origin of the mother, province of residence, apparent temperature, infant's sex, and gestational age.

Chapter 6

Dairy cattle mortality as a sentinel for the effects of air pollution on human health

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In preparation

Abstract

Objective

Air pollution is a trigger for human mortality. The use of animal sentinels as models for epidemiologic studies of environmental exposures and human diseases might improve causal inference. This is the first study investigating the role of ozone (O_3), particulate matter (PM_{10}) and nitrogen dioxide (NO_2) on the risk of mortality in an animal population.

Methods

We used air pollution concentrations at the level of the municipality for a total of 87,108 dairy cow deaths from 2006 to 2009. We combined a case-crossover design with distributed lag nonlinear models (DLNM) to assess the potential delayed effects of air pollution on cattle mortality up to 25 days after exposure, while controlling for weather effects. Separate models were developed for the warm (April – September) and the cold period (October – March) of the year.

Results

We found acute and delayed effects of air pollution on dairy cattle mortality during the warm season. A 10 $\mu g/m^3$ increase in 2-day (lag 0–1) O_3 exposure was associated with a 1.3% (95% CI 0.3 to 2.2) increase in the risk of mortality. The increase in mortality for a 10 $\mu g/m^3$ increase in same-day (lag 0) PM_{10} or NO_2 exposure was 1.2% (95% CI -0.3 to 2.8) and 9.4% (95% CI 6.4 to 12.4), respectively. Compared with the acute effects, the cumulative 26-day (lag 0 to 25) estimates were considerably larger for O_3 (3.6%, 95% CI 0.4 to 6.9) and PM_{10} (5.1%, 95% CI 0.8 to 9.5), but not for NO_2 (2.9%, 95% CI -4.1 to 10.3). Results are unchanged using unconstrained distributed lag models. We did not find consistent evidence for air pollution effects during the cold period.

Conclusions

Our study in cattle adds to the existing epidemiological findings in human and further improves its causality. Furthermore, our results indicate that the observed effects are not due primarily to short-term mortality displacement. Compared with human studies, we observed higher mortality risk associated with air pollution among cattle, suggesting that dairy cows may be sensitive indicators of air pollution and provide an early warning system for public health intervention.

Introduction

Studies on the effects of air pollution on animal mortality are limited, although already in the 1870s, death of cattle during a livestock show in England was associated with a dense industrial fog.[1] Also in Belgium cattle died in the fog of 1911,[2] and during the Meuse valley air pollution episode in 1930.[3] Such episodes initiated successful efforts and legislation to reduce air pollution. Nevertheless, even at current concentration levels, the relation between air pollution and excess morbidity and mortality still exists.

Numerous studies reported that daily variations in human morbidity and mortality are associated with daily variations in the levels of air pollution.[4] However, still some debate exists about the lag period associated with these exposures, which may include both harvesting and delayed effects, contributing differently to the net cumulative effects. The harvesting hypothesis states that short-term increases in air pollution simply shorten the life span of frail individuals, implying a short-term positive correlation between exposure and daily deaths, followed by a deficit in mortality at longer lags.

The potential health effects of environmental factors on livestock is not only of concern for animal welfare and health,[5] but also implies economic losses.[6] Moreover, studies on cattle mortality can corroborate or inform epidemiologic studies in humans. The advantages of using animals as sentinels or comparative models of human disease accrue in part from their relative freedom from concurrent exposures, bias due to confounding, and, to some extent, exposure misclassification.[7] Dairy cows have a relatively long life span, limited population variability in lifestyle and dietary habits, limited geographical mobility[8] and (partial) outdoor housing. Moreover, in many countries farm animals are subject to a stringent mandatory registration procedure from birth till death, which is at the individual level for ruminants.

In this study, we investigated the effect of ozone (O_3), particulate matter with diameter less than 10 $\mu g/m^3$ (PM_{10}), and nitrogen dioxide (NO_2) on mortality among dairy cows in Belgium. We applied a case-crossover design and accounted for harvesting and delayed exposure effects by using distributed lag non-linear models (DLNM).[9 10] A DLNM has the advantage of providing cumulative effects of

air pollution by flexibly estimating contributions at different lag times, thus accounting for delayed effects and short-term harvesting.[11]

Methods

Data

Data on cattle mortality were extracted from Sanitel, a national-level computerized database for the registration and traceability of farm animals, managed by the Federal Agency for the Safety of the Food Chain.[12] For all adult dairy cows (≥2 years) that died (different from culling in slaughterhouse) in Belgium during the period 2006-2009, we obtained information on date of birth and death, farm identification and postal code. The majority of dairy cows in Belgium are of the Holstein Friesian breed.

Daily 8-hour maximum O_3 concentrations and daily average PM_{10} and NO_2 concentrations were obtained from the Belgian Interregional Environment Agency. For its assessment, a dense network of automatic monitoring sites, as implemented by the three Belgian regions, collected real-time data on a half-hourly basis. The average distance between the nearest measuring stations is about 25 km. Data from monitoring stations are combined with land cover data obtained from satellite images in a spatial-temporal (Kriging) interpolation model, described by Janssen *et al*.[13] This provides interpolated air pollutant concentration estimates on a 4 x 4 km^2 grid, which are then used to calculate population-weighted averages per municipality. Previous studies suggest that PM_{10} estimates correlate well with individual exposure, as assessed by carbon load in human macrophages.[14] Air pollution levels are linked to cattle mortality data through the postal code of the farm.

Because climate is a known confounder of the association between air pollution and health,[15 16] data on mean air temperature and average relative humidity were provided by the Belgian Royal Meteorological Institute (KMI). We used data from one central and representative station in Uccle (Brussels), because Belgium is very uniform for temperature, as a result of extremely small altitudinal and latitudinal gradients: elevations range from 0 to 694 m above sea level, and the distance between the northernmost and southernmost part is only 224 km.

Statistical analyses

The case-crossover design is widely used for analyzing short-term exposures with acute outcomes.[17-19] It is a variant of the matched case-control study, where each subject serves as its own control so that known and unknown time-invariant confounders are inherently adjusted for by study design.[20] This design samples only cases (deaths in this study) and compares each subject's exposure in a time period just before a case event (the hazard period) with that subject's exposure at other times (the control periods). Selection bias was avoided by applying a bidirectional time-stratified design.[21] Control days are taken from the same calendar month and year as the case day (*i.e.* day of death), both before and after the case, thus controlling for long-term trends and season by design. Cases and controls were additionally matched by day of the week to control for any weekly patterns in deaths or pollution.

We combined the case-crossover design with distributed lag non-linear models (DLNM) to account for potential harvesting and delayed effects of air pollution on dairy cow mortality. This study applies recent extensions of the DLNM methodology beyond aggregated time series data,[22] specifically implementing them in a conditional logistic regression model with individual-level exposure measures. The DLNM is defined through a "cross-basis" function, which allows the simultaneous estimation of a non-linear exposure-response association and non-linear effects across lags, the latter termed lag-response association. In addition to a cross-basis for the air pollutant, we also included a cross-basis for mean temperature in the model to account for the (potentially delayed) effects of heat and cold on mortality. In both cross-bases, the maximum lag was set to 25 days and natural cubic splines with 6 degrees of freedom (df) were used to model the lag structure. The knots in the lag space were set at equally spaced values on the log scale of lags to allow more flexible lag effects at shorter delays.[11] We assumed a linear exposure-response shape for the air pollutants, whereas the temperature-mortality association was modelled with a natural cubic spline with 5 df. Spline knots were placed at equal spaces in the temperature range to allow enough flexibility in the two ends of the temperature distribution.

We developed separate models for each of three air pollutants within the warm (April-September) and the cold (October-March) period of the year, as human

studies have found effect modification by season.[16] Models were additionally adjusted for the moving average of humidity on the current day and the previous day (lag 0–1), using a natural cubic spline with 3 degrees of freedom. We calculated relative risks (RR) of mortality for a 10 μg/m^3 increase in air pollutant concentrations. Reported estimates, computed as the overall cumulative risk accounting for the 0–25 lag period, are presented as percent change in mortality with corresponding 95% CIs.

In sensitivity analyses we used an unconstrained distributed lag model to define the lag structure, that is, a model in which each lag is entered as a separate variable.[23 24] Because of the correlation between air pollution concentrations on days close together, the unconstrained distributed lag model will result in unstable estimates for the individual lags, but it is known as more flexible and less prone to bias for the estimate of the overall effect.[23] We also investigated the potential influence of Bluetongue disease on the observed results. There were two outbreaks of Bluetongue Virus Serotype 8 in Belgium within the study period: from August to December 2006 and from July to December 2007.[25] Because the spread of Bluetongue in Belgium has been found to be associated with weather conditions[26] and because of the correlation between meteorology and air pollution, we examined whether our results were robust to the exclusion of these epidemics from the analyses by using both constrained (as in the main analyses) and unconstrained distributed lag models.

All analyses were performed with the statistical software R (R Foundation for Statistical Computing, Vienna, Austria) using the "dlnm" package.[11]

Results

Data description

There were 87,108 cow deaths in Belgium from 2006 to 2009. Table 1 shows descriptive statistics for daily mortality, air pollutants and weather variables. In the warm season there were on average 55 cases per day and in the cold season 65 cases. The average concentrations of O_3, PM_{10} and NO_2 in the warm season were 80.6 μg/m^3, 25.4 μg/m^3 and 15.9 μg/m^3 respectively, whereas the corresponding concentrations in the cold season were 47.6 μg/m^3, 27.8 μg/m^3

and 22.6 µg/m^3 respectively. To highlight sufficient variation around a non-zero mean value as suggested in case-crossover studies,[27] table 1 also presents the "relevant exposure term" which is the absolute difference between each pollutant's levels on the case day and its average concentrations over the control days.

Table 1. Summary statistics for daily cattle mortality, weather conditions and air pollution levels and for the absolute differences between the daily levels of each pollutant (case days) and the average concentrations over the control days, Belgium 2006-2009.

			Percentiles						
Season	**Variable**	**Mean**	**1st**	**5th**	**25th**	**50th**	**75th**	**95th**	**99th**
Warm	**Daily number of cow deaths**								
(n=39,979)		54.6	18.0	23.0	38.0	53.0	68.5	96.0	116.0
	Exposure on case days								
	O_3 (µg/m^3)	80.6	33.1	45.2	62.9	77.2	92.8	130.6	159.7
	PM_{10} (µg/m^3)	25.4	8.1	11.4	16.9	22.3	31.4	48.3	64.1
	NO_2 (µg/m^3)	15.9	2.5	4.7	9.9	14.7	20.5	31.0	39.3
	Temperature (°C)	15.6	5.2	8.2	13.0	15.7	18.3	22.3	25.0
	Humidity (%)	69.9	39.5	48.7	63.6	70.3	78.1	86.1	91.2
	Exposure difference between case days and average over control days								
	O_3 (µg/m^3)	18.2	0.3	1.2	6.2	14.0	25.6	49.9	68.2
	PM_{10} (µg/m^3)	8.6	0.1	0.5	2.9	6.5	12.0	23.6	37.3
	NO_2 (µg/m^3)	4.8	0.1	0.3	1.8	3.8	6.7	13.0	18.4
Cold	**Daily number of cow deaths**								
(n=47,129)		64.6	12.0	24.0	45.0	65.0	83.0	107.0	126.0
	Exposure on case days								
	O_3 (µg/m^3)	47.6	7.4	12.8	32.1	49.0	62.5	79.8	88.8
	PM_{10} (µg/m^3)	27.8	5.5	9.5	16.6	23.4	34.4	59.8	90.1
	NO_2 (µg/m^3)	22.6	3.5	6.6	13.8	21.2	29.5	43.7	57.9
	Temperature (°C)	6.2	-3.2	-1.0	3.1	6.6	9.1	13.4	15.4
	Humidity (%)	81.0	50.8	63.3	77.0	81.9	87.2	92.5	95.1
	Exposure difference between case days and average over control days								
	O_3 (µg/m^3)	14.6	0.3	1.2	6.0	12.5	21.3	34.9	45.7
	PM_{10} (µg/m^3)	13.2	0.1	0.9	4.6	9.8	17.9	36.8	62.3
	NO_2 (µg/m^3)	8.6	0.1	0.6	3.2	6.8	11.9	22.8	33.0

Abbreviations: O_3, ozone; PM_{10}, particulate matter with diameter less than 10 µg/m^3; NO_2, nitrogen dioxide

Linear correlation coefficients between air pollutants and meteorological variables are presented in Table 2. Correlations were highest between PM_{10} and NO_2 (linear correlation coefficient (r) around 0.7 in both seasons) and between O_3 and NO_2 (only in the cold season, r=-0.64). The correlation between PM_{10} and O_3 was strongest in the cold season (r=-0.50, compared with r=0.33 in the warm season). O_3 was positively correlated with temperature in both seasons (warm

season, r=0.41; cold season, r=0.31), whereas PM_{10} and NO_2 were negatively correlated with temperature in the cold season (r=-0.33 and r=-0.43 respectively).

Table 2. Matrix of linear correlation coefficients between air pollutants and weather variables, Belgium 2006-2009*

Season	Exposure	O_3	PM_{10}	NO_2	Temperature	Humidity
Warm	O_3	1				
(n=39,979)	PM_{10}	0.33	1			
	NO_2	0.11	0.68	1		
	Temperature	0.41	0.10	-0.05	1	
	Humidity	-0.66	-0.29	-0.24	-0.31	1
Cold season	O_3	1				
(n=47,129)	PM_{10}	-0.50	1			
	NO_2	-0.64	0.74	1		
	Temperature	0.31	-0.33	-0.43	1	
	Humidity	-0.40	-0.09	-0.01	-0.11	1

Abbreviations: O_3, ozone; PM_{10}, particulate matter with diameter less than 10 $\mu g/m^3$; NO_2, nitrogen dioxide
*P<0.001 for all correlations

DLNM analyses

During the warm season, highest relative risks were observed on the day of exposure (PM_{10} and NO_2) or the day after (O_3), immediately followed by a 2- to 3-day deficit in mortality (Figure 1). Acute effects were significant for O_3 and NO_2, but not for PM_{10}. For O_3 and PM_{10}, the deficit in mortality was followed by a significantly increased risk lasting for one (O_3) to two (PM_{10}) weeks. The lag structure in the cold season was less consistent. Mortality was significantly increased 3 to 10 days after O_3 exposure and 17 to 22 days after PM_{10} exposure.

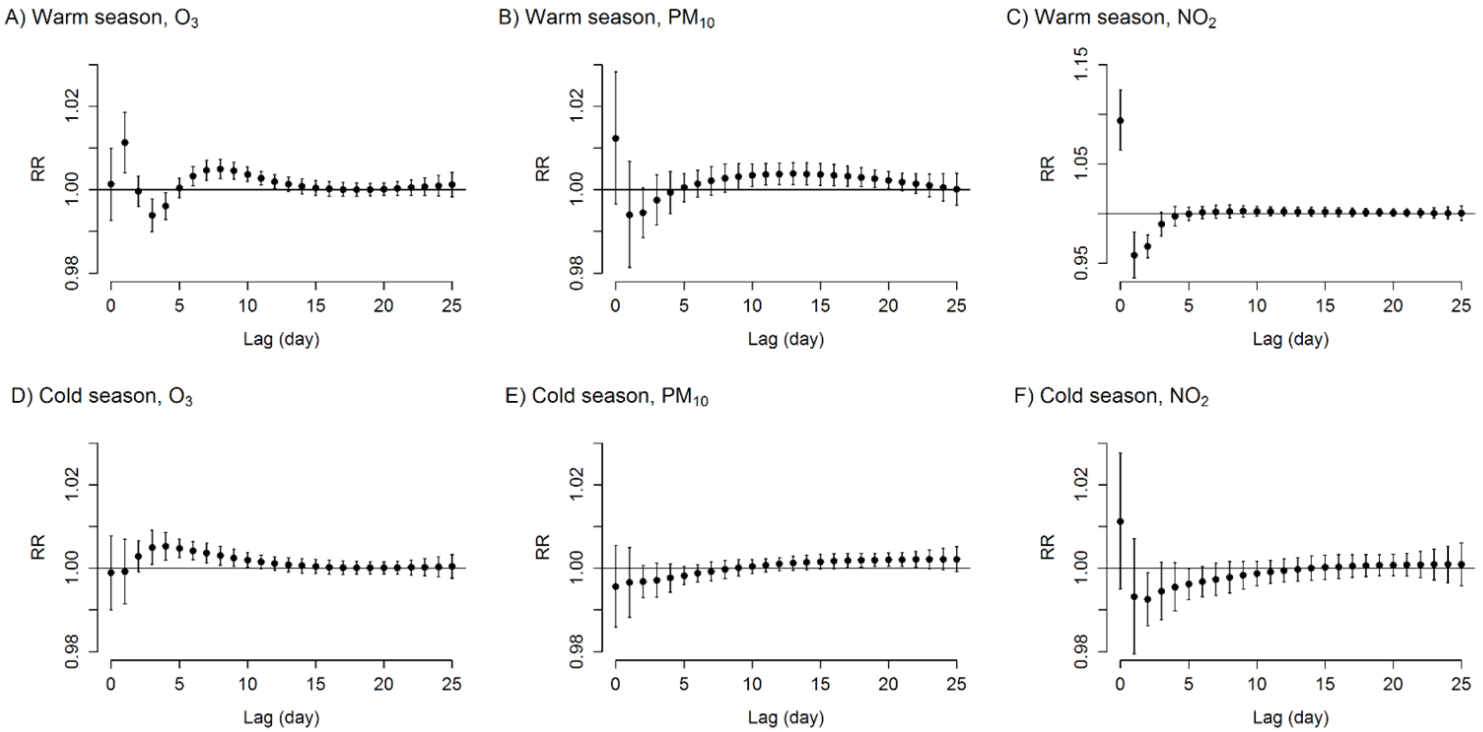

Figure 1. Lag-specific relative risks (RR, with 95% CIs) of dairy cow mortality for a 10 µg/m^3 increase in air pollutant concentrations during the warm season (A, B and C for O_3, PM_{10}, and NO_2 respectively) and the cold season (D, E and F for O_3, PM_{10}, and NO_2 respectively), Belgium 2006-2009

Overall cumulative effects of air pollutants on dairy cow mortality are presented in Table 3. The increase in the risk of mortality for a 10 µg/m^3 increase in air pollutant concentration was 1.3% (95% CI 0.3 to 2.2) for O_3 (lag 0–1), 1.2% (95% CI -0.3 to 2.8) for PM_{10} (lag 0), and 9.4% (95% CI: 6.4 to 12.4) for NO_2 (lag 0). The overall 26-day estimates, incorporating the harvesting and delayed effects, were considerably larger than the acute effects for O_3 (3.6%, 95% CI 0.4 to 6.9) and PM_{10} (5.1%, 95% CI 0.8 to 9.5), but not for NO_2 (2.9%, 95% CI -4.1 to 10.3). Sensitivity analyses for the warm season gave similar results. Both the use of the unconstrained distributed lag model and the exclusion of the Bluetongue epidemics from the study period resulted in slightly higher estimates (supplementary Table S1).

We did not find consistent evidence for acute or delayed effects of air pollution exposure on dairy cow mortality in the cold season (Table 3). Although we observed a significant cumulative 26-day effect of O_3 in the main analysis (3.9%, 95% CI 0.9 to 6.9) and in the unconstrained distributed lag model, this effect disappeared after excluding the epidemic Bluetongue periods from the models (supplementary Table S2).

Table 3. Cumulative effects of air pollution on dairy cow mortality along the lag days, Belgium 2006-2009. Estimates represent the percent change in dairy cow mortality for a 10 µg/m^3 increase in air pollutant concentration.

		Percent change (95% CI)					
Season	**Lag (day)**	**O_3**		**PM_{10}**		**NO_2**	
Warm	0	0.1	(-0.7 to 1.0)	1.2	(-0.3 to 2.8)	9.4	(6.4 to 12.4)*
(n=39,979)	0–1	1.3	(0.3 to 2.2)*	0.6	(-0.9 to 2.1)	4.8	(1.8 to 7.8)*
	0–25	3.6	(0.4 to 6.9)*	5.1	(0.8 to 9.5)*	2.9	(-4.1 to 10.3)
Cold	0	-0.1	(-1.0 to 0.8)	-0.4	(-1.4 to 0.5)	1.1	(-0.5 to 2.8)
(n=47,129)	0–1	-0.2	(-1.1 to 0.7)	-0.8	(-1.7 to 0.1)	0.4	(-1.2 to 2.1)
	0–25	3.9	(0.9 to 6.9)*	0.6	(-2.2 to 3.6)	-2.2	(-7.0 to 2.8)

Abbreviations: O_3, ozone; PM_{10}, particulate matter with diameter less than 10 µg/m^3; NO_2, nitrogen dioxide
*P<0.05

Discussion

Despite the recognition that animals could be useful sentinels for human health risks, the full potential of linking animal and human health information to provide warning of such shared risks from environmental hazards has not been realized.[28] Possible reasons for this include the professional segregation of human and animal health communities, the separation of human and animal surveillance data, and evidence gaps in the linkages between human and animal responses to environmental health hazards.

Day-to-day variations in air pollution are weather-driven and have been linked to daily fluctuations in hospital admissions and mortality in numerous human epidemiological studies. To the best of our knowledge, this is the first epidemiological study that uses an animal population to investigate short-term variations in mortality in association with recent exposure to air pollution. We found significant increases in the risk of dairy cattle mortality associated with air pollution, both particulate (PM_{10}) and gaseous (O_3 and NO_2), during the warm period of the year. Exposure to NO_2 was associated with a same-day increase in mortality, but the overall cumulative 26-day effect accounting for harvesting was not significant. For O_3 and PM_{10}, however, the overall (lag 0–25) effects are substantially larger than the acute effects (up to lag 0–1), indicating that the adverse response to pollution persists up to some weeks after the exposure.

Whereas it has been argued that risk assessments using relatively short timescales might have overestimated the public health impact of air pollution because of harvesting, our analysis indicates that such studies might have underestimated the total effects. This was also suggested by some other studies that have examined the temporal pattern of the association using longer follow-up periods.[24 29-32] As in our study, they found extended effects of O_3[29] and particulates,[24 30-32] with larger estimates for the overall cumulative effect than for the acute effects. As pointed out by Zanobetti *et al.*,[24] numerous epidemiologic studies have shown that air pollution is associated with exacerbation of illness, which might result in an increased recruitment into the pool of individuals at risk of dying. This may occur at different lags, depending on the mechanism and individual, and at a slower pace than death out of the risk pool, which might result in delayed increases in mortality persisting for several days or weeks. Extended

air pollution effects on mortality are supported by evidence from the historical London smog episode in 1952, which was followed by elevated mortality rates up to 3 months after the exposure.[33]

We did not find consistent evidence for an association between air pollution and dairy cattle mortality during the cold period of the year, indicating that the triggering effect of air pollution is not equally harmful under different weather conditions, even after strong adjustment for immediate and delayed effects of outdoor temperature. This finding is consistent with results from a study on human mortality in the northern part of Belgium (Flanders), reporting much larger effects of particulates during the warmer period of the year.[16] Stronger associations between air pollution and daily mortality in the warm season have also been found in other human studies.[34-37]

We can only speculate about the mechanisms underlying the effect modification by season. The difference cannot be explained by differences in concentration levels, because concentrations of both PM_{10} and NO_2 were higher and more variable in the cold season (Table 1). A possible explanation is the large difference in the time spent outdoor between both seasons: free-ranging cows are, apart from the daily milking moments, the majority of their time on pasture from March-May until October, whereas they are in general continuously in the stable during the other months of the year. However, correlations between indoor and outdoor exposure in the cold season as high as 0.79 have been reported,[38] and correlations might be even higher for (relatively open) stables. As studies have shown a higher inflammatory activity of particulate matter (PM) in the warm season,[39 40] our results might also be due to seasonal differences in air pollution mixture or composition of PM.

Despite physiological and anatomical differences between cattle and human, biological changes in response to air pollution exposure are expected to be similar for both species. Compared with large-scale studies on human populations, which typically have examined the risk of mortality up to only a few days after exposure, our estimates for the immediate effects of O_3 and NO_2 during the warm season are considerably larger. Assuming that 10-ppb O_3 equals 20 $\mu g/m^3$ and converting the 8-hour maximum concentration to the daily average,[41] our estimate corresponds to a 4.8% increase in mortality for a 10-ppb increase in daily average

O_3. Estimates from meta-analyses range from 0.87% to 1.37% per 10-ppb in daily average O_3,[41-46] and those from multi-city studies range from 0.25% to 1.43%.[47-50] Meta-analytic and multi-city estimates for a 10 µg/m^3 increase in NO_2 range from 0.13% to 1.23%,[43 47 50 51] whereas corresponding estimates for PM_{10} range from 0.2% to 0.6%.[43 44 47 50 52 53]

Considering the medium-term effects, our result for the net effect of 8-hour maximum O_3 during the warm season (3.6% for a 10 µg/m^3 increase) is close to the estimate for respiratory mortality obtained by combining data from 21 European cities.[29] The multi-city study found a 21-day increase in respiratory deaths of 3.35% (95% CI 1.90 to 4.83) for each 10 µg/m^3 increase in O_3, whereas effects on total and cardiovascular mortality were only found in summer and were counterbalanced by negative effects thereafter. Also our overall estimate for PM_{10} (5.1% for a 10 µg/m^3 increase) is closer to results reported for respiratory mortality than those reported for total or cardiovascular mortality. Based on data from 10 European cities, Zanobetti *et al.*[32] investigated the effect of PM_{10} on deaths up to 40 days after the exposure and found a 4.2% (95% CI 1.08 to 7.42) increase in respiratory deaths and a 1.97% (95% CI 1.38 to 2.55) in cardiovascular deaths for each 10 µg/m^3 increase in PM_{10}. Similarly, the 41-day increase in mortality associated with a 10 µg/m^3 increase in black smoke in Dublin was 3.6% (95% CI.0 to 4.3) for respiratory mortality, but only 1.1% (95% CI 0.8 to 1.3) for total mortality.[31] The 41-day increased risk of total mortality associated with a 10 µg/m^3 increase in PM_{10} estimated for 10 European cities was 1.61% (95% CI 1.02 to 2.20).[30]

The difference in effect estimates between dairy cattle and human studies is remarkable. However, cattle are known to be susceptible for respiratory disease because of their small physiological gaseous exchange capacity, greater basal ventilatory activity, and greater anatomical compartmentalization of the lung as compared with other mammals.[54] This makes bovine lungs highly susceptible to bacterial infection and lung damage, which might explain the larger impact of air pollution. Also the smaller population variability among dairy cattle might have contributed to the larger estimates observed in this animal population.

Common to other epidemiologic studies, the first limitation of our study is that it cannot prove causality and is likely to suffer from bias because of its observational

nature. However, environmental epidemiologic studies on animals are expected to be relatively free from confounding factors such as time spent outdoors, use of air conditioning, housing construction, occupational exposures, cigarette smoking and alcohol consumption.[7] A second limitation is that we derived daily average levels of weather variables and air pollutants from outdoor monitoring stations, so that the individual exposure levels may have differed substantially from the estimated exposure. However, the restricted daily mobility and lower frequency of migration compared to humans contribute to the likelihood that exposure assessment can be conducted more accurately in studies of animal diseases. Whereas human in Western countries spend a lot of time indoor, the majority of adult dairy cattle are on pasture during summer, making outdoor exposure a better proxy for actual individual cow exposure. Moreover, in this study the case-crossover design represents an attractive alternative to Poisson models to investigate the acute effects of an exposure, because of the possibility to use individual-level information on exposures.

Conclusion

This is the first epidemiological study demonstrating that day-to-day variations in animal mortality are associated with day-to-day variations in ambient air pollution. In addition, this study provides further evidence that acute exposures have long lasting effects. Results are consistent with those from human studies, although effects estimates are found to be higher. Dairy cows are expected to have similar biological responses to environmental exposures as humans, but they have limited geographic mobility, are relatively free from confounding and may be exposed to higher concentrations than human. Therefore, they may be sensitive indicators of environmental hazards and provide an early warning system for public health interventions.

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Supplementary material

Table S1. Cumulative effects of air pollution on dairy cow mortality along the lag days during the warm season, Belgium 2006-2009, for the total study period (main analysis) and excluding the Bluetongue epidemics (August-December 2006 and July-December 2007), and using a natural cubic spline with 6 degrees of freedom (main analysis) and an unconstrained distributed lag to model the lag structure. Estimates represent the percent change (with 95% CI) in dairy cow mortality for a 10 µg/m^3 increase in air pollutant concentration.

		Percent change (95% CI)			
	Lag	Total study period		Excluding Bluetongue epidemics	
Exposure	(day)	Natural spline	Unconstrained	Natural spline	Unconstrained
O_3	0	0.1 (-0.7 to 1.0)	0.3 (-0.6 to 1.2)	0.0 (-0.9 to 1.0)	0.2 (-0.8 to 1.2)
	0–1	1.3 (0.3 to 2.2)*	1.4 (0.3 to 2.5)*	1.2 (0.1 to 2.2)*	1.6 (0.4 to 2.8)*
	0–25	3.6 (0.4 to 6.9)*	3.8 (0.5 to 7.1)*	3.4 (-0.2 to 7.0)	4.0 (0.4 to 7.7)*
PM_{10}	0	1.2 (-0.3 to 2.8)	0.7 (-1.1 to 2.5)	1.5 (-0.2 to 3.3)	0.7 (-1.3 to 2.7)
	0–1	0.6 (-0.9 to 2.1)	-0.1 (-2.0 to 1.8)	0.4 (-1.3 to 2.1)	-0.6 (-2.8 to 1.6)
	0–25	5.1 (0.8 to 9.5)*	5.7 (1.2 to 10.3)*	5.6 (0.9 to 10.6)*	5.8 (0.9 to 11.0)*
NO_2	0	9.4 (6.4 to 12.4)*	10.0 (6.7 to 13.3)*	9.5 (6.2 to 12.9)*	9.5 (5.9 to 13.2)*
	0–1	4.8 (1.8 to 7.8)*	4.9 (1.4 to 8.5)*	4.6 (1.3 to 8.0)*	4.9 (1.0 to 9.0)*
	0–25	2.9 (-4.1 to 10.3)	3.1 (-4.1 to 10.8)	3.9 (-3.7 to 12.1)	4.3 (-3.6 to 12.8)

Abbreviations: O_3, ozone; PM_{10}, particulate matter with diameter less than 10 µg/m^3; NO_2, nitrogen dioxide

*P<0.05

Table S2. Cumulative effects of air pollution on dairy cow mortality along the lag days during the cold season, Belgium 2006-2009, for the total study period (main analysis) and excluding the Bluetongue epidemics (August-December 2006 and July-December 2007), and using a natural cubic spline with 6 degrees of freedom (main analysis) and an unconstrained distributed lag to model the lag structure. Estimates represent the percent change (with 95% CI) in dairy cow mortality for a 10 μg/m^3 increase in air pollutant concentration.

		Percent change (95% CI)			
	Lag	**Total study period**		**Excluding Bluetongue epidemics**	
Exposure	**(day)**	**Natural spline**	**Unconstrained**	**Natural spline**	**Unconstrained**
O_3	0	-0.1 (-1.0 to 0.8)	-0.4 (-1.4 to 0.6)	-0.4 (-1.4 to 0.6)	-0.7 (-1.8 to 0.4)
	0–1	-0.2 (-1.1 to 0.7)	0.1 (-1.0 to 1.2)	-1.0 (-2.1 to 0.0)	-1.0 (-2.3 to 0.3)
	0–25	3.9 (0.9 to 6.9)*	3.2 (0.3 to 6.2)*	1.4 (-1.8 to 4.7)	0.4 (-2.8 to 3.7)
PM_{10}	0	-0.4 (-1.4 to 0.5)	0.3 (-0.9 to 1.5)	-0.1 (-1.2 to 1.0)	0.1 (-1.2 to 1.4)
	0–1	-0.8 (-1.7 to 0.1)	-0.6 (-1.8 to 0.6)	-0.4 (-1.5 to 0.6)	0.3 (-1.0 to 1.7)
	0–25	0.6 (-2.2 to 3.6)	1.6 (-1.3 to 4.6)	2.5 (-0.8 to 6.0)	4.1 (0.7 to 7.6)*
NO_2	0	1.1 (-0.5 to 2.8)	2.4 (0.6 to 4.3)*	1.2 (-0.6 to 3.0)	2.3 (0.2 to 4.5)*
	0–1	0.4 (-1.2 to 2.1)	-0.7 (-2.6 to 1.4)	1.0 (-0.9 to 2.9)	0.0 (-2.2 to 2.4)
	0–25	-2.2 (-7.0 to 2.8)	-0.6 (-5.6 to 4.5)	0.6 (-4.8 to 6.3)	2.4 (-3.1 to 8.3)

Abbreviations: O_3, ozone; PM_{10}, particulate matter with diameter less than 10 μg/m^3; NO_2, nitrogen dioxide
*P<0.05

Chapter 7

Dairy cattle mortality as a sensitive warning system for the effects of high and low ambient temperatures on human health

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In preparation

Abstract

Objective

Extreme ambient temperatures are associated with an increased risk of mortality among humans, which is a concern because of the predicted climate change. The use of animal sentinels as models for epidemiologic studies of human diseases and environmental exposures might add to the available evidence and might provide additional insights. Therefore, we investigated the temporal pattern of the effects of low and high ambient temperatures on the risk of mortality among dairy cattle.

Methods

We applied a case-crossover design combined with distributed lag nonlinear models (DLNM) to assess the nonlinear and delayed effects of temperature on dairy cattle mortality. We used quasi-Poisson models on 87,108 dairy cow deaths in Belgium from 2006 to 2009, stratifying by the warm (April-September) and the cold (October-March) season of the year.

Results

During the warm season high as well as low temperatures were associated with significantly increased mortality risk among dairy cattle. Heat effects were acute and were followed by a deficit in mortality three to five days after the exposure. The estimated 3-day (lag 0–2) increase in dairy cattle mortality for a 1°C increase in mean temperature above the heat threshold (16.8°C) was 3.16% (95% CI 1.64 to 4.71). Taking into account the harvesting effect, the corresponding heat estimate at lag 0–10 was 2.17% (95% CI 0.11 to 4.28). Cold effects were delayed by five days and persisted up to 18 days after the exposure. Over lag 0–25 days, the estimated increase in mortality for a 1°C decrease below the cold threshold (13.9°C) was 4.90% (95% CI 0.40 to 9.60). We did not find evidence for temperature effects during the cold season.

Conclusions

Consistent with epidemiological observations in humans, extreme temperatures are associated with an increased mortality risk among dairy cattle. Dairy cows may be sensitive indicators of environmental hazards and provide an early warning system for public health intervention.

Introduction

It is well recognized that in developed countries, the major health consequences of climate change will be due to extreme weather events.[1] Daily variations in human morbidity and mortality are associated with daily variations in ambient temperature, with increased health risks at both ends of the temperature distribution.[2-4]

Also farm animals are known to suffer from temperature extremes. Heat stress reduces feed intake, milk yield, growth rate and reproductive performance, which lead to major economic losses to the dairy farmers.[5-7] Therefore, thermal stress in dairy cattle has mainly been investigated because of economic reasons[8] and to guide management strategies.[9] The effect of temperature on dairy cattle death rates has received less attention. Some studies have documented increases in dairy cattle mortality associated with high temperatures,[10-13] and few have tried to identify critical heat thresholds.[14 15] As dairy cattle tend to be much more tolerant for low than for high temperatures,[16 17] effects of cold have not been investigated much. Nevertheless, few studies have reported cold-related decreases in milk yield[18] and increases in mortality.[13]

Despite the recognition that animals could be useful sentinels for human health risks, the full potential of linking animal and human health information to provide warning of such shared risks from environmental hazards has not been realized.[19] Reasons appear to include the professional segregation of human and animal health communities, the separation of human and animal surveillance data, and evidence gaps in the linkages between human and animal responses to environmental health hazards.[19] Animal populations such as dairy cows might serve as useful sentinel models for human disease, as they are expected to be less subject to concurrent exposures, bias due to confounding, and exposure misclassification than human populations.[20]

Therefore, we investigated the association between day-to-day variations in ambient temperature and day-to-day variations in dairy cattle deaths in Belgium. We used a case-crossover design and considered the possibility of both non-linear and delayed temperature effects by using distributed lag non-linear models (DLNM).[21 22] A DLNM has the advantage of providing cumulative effects of temperature by flexibly estimating contributions at different lag times, thus

accounting for delayed effects and short-term mortality displacement (harvesting).[23]

Methods

Data

Data on cattle mortality were extracted from Sanitel, a national-level computerized database for the registration and traceability of farm animals, managed by the Federal Agency for the Safety of the Food Chain.[24] For all adult dairy cows (≥2 years) that died (different from culling in slaughterhouse) in Belgium during the period 2006-2009, we obtained information on date of birth and death, farm identification and postal code, and herd size at the moment of data extraction. The majority of dairy cows in Belgium are of the Holstein Friesian breed.

Meteorological data consisting of daily mean air temperature and average relative humidity were provided by the Belgian Royal Meteorological Institute. We used data from a central and representative station in Uccle (Brussels), because Belgium is very uniform for temperature, as a result of extremely small altitudinal and latitudinal gradients: elevations range from 0 to 694 m above sea level, and the distance between the northernmost and southernmost part is only 224 km.

As ambient air pollution levels might confound the association between temperature and mortality, we obtained daily air pollution concentrations from the Belgian Interregional Environment Agency. We considered ozone (O_3, 8-hour maximum values), particulate matter with diameter less than 10 µm (PM_{10}, daily averages), and nitrogen dioxide (NO_2, daily averages). In Belgium, air pollution is measured by a dense network of automatic monitoring sites, collecting real-time data on a half-hourly basis. The average distance between the nearest measuring stations is about 25 km. Daily air pollution concentrations at the level of the municipality are obtained by a spatial-temporal (Kriging) interpolation model that combines data from monitoring stations with land cover data obtained from satellite images.[25] Daily average concentrations for Belgium were then calculated by weighing the municipality-specific concentrations by the number of animals (herd size at the moment of data extraction) per municipality.

Statistical analysis

The association between ambient temperature and dairy cattle mortality was investigated by using a case-crossover design, which is widely used for short-term exposures and acute outcomes.[26] It is a variant of the matched case-control study, where each subject serves as its own control so that known and unknown time-invariant confounders are inherently adjusted for by study design.[27] We used the bidirectional time-stratified design to avoid selection bias:[28] control days were taken from the same calendar month and year as the case day (i.e. day of death), both before and after the case, thus controlling for long-term trends and season by design. Cases and controls were additionally matched by day of the week to control for any weekly patterns in deaths.

When all subjects have a common (national-level) exposure, the case-crossover using conditional logistic regression is a special case of time-series analysis.[29] Data can be aggregated into daily counts and a Poisson model with stratum indicators gives identical estimates to those from conditional logistic regression. Conditional Poisson models have some advantages over conditional logistic models: they are computationally less intensive and they can allow for overdispersion or auto-correlation in the original counts.[30] Despite these advantages, they are little used.[30] In this study, we used conditional quasi-Poisson models that allow for overdispersion in daily deaths. Analyses were stratified by warm (April-September) and cold (October-March) season because free-ranging cows are, apart from the daily milking moments, the majority of their time on pasture during the warm season, whereas they are mostly in the stable during the cold season.

To account for potential nonlinear and delayed effects of temperature, we combined the quasi-Poisson model with a distributed lag non-linear model (DLNM).[21 22] The DLNM is defined through a "cross-basis" function, which allows simultaneous estimation of the non-linear exposure-response association and the non-linear effects across lags, the latter termed lag–response association. The maximum lag was set to 25 days. First we applied a "natural cubic spline–natural cubic spline" DLNM. We used a natural cubic spline with 4 degrees of freedom (df) to model the temperature–mortality association and a natural cubic spline with 6 df to model the lagged effect. Spline knots were placed at equal spaces in the

temperature range to allow enough flexibility in the two ends of the temperature distribution. The knots in the lag space were set at equally spaced values on the log scale of lags to allow more flexible lag effects at shorter delays.[23] Season-specific median temperatures were used as the reference value to calculate relative risks.

When the temperature–mortality curve appeared to be U-shaped, we used a "double threshold–natural cubic spline" DLNM, assuming the effect of high temperature is linear above the heat threshold, whereas the effect of low temperature is linear below the cold threshold. We tested multiple heat and cold thresholds and searched for the combination that minimized the residual deviance of the model. We then estimated the relative risks of mortality for a 1°C increase in temperature above the heat threshold and a 1°C decrease below the cold threshold. All models were adjusted for the moving average of humidity on the current day and the previous days (lag 0–1), using a natural cubic spline with 3 df. We used the Akaike information criterion for quasi-Poisson models (Q-AIC) to compare the fit of models.[22 31]

We ran several sensitivity analyses. First we used an unconstrained distributed lag model to define the lag structure, that is, a model in which each lag is entered as a separate variable.[32 33] Because of the correlation between temperatures on days close together, the unconstrained distributed lag model will result in unstable estimates for the individual lags, but it is known as more flexible and less prone to bias for the estimate of the overall effect.[32] In a second sensitivity analysis, we accounted for the potentially confounding effects of air pollution on the temperature-mortality association by adding a crossbasis for each air pollutant at a time. For each air pollutant, the maximum lag was set at 25 and we used a linear function to model the exposure–response association and a natural cubic spline with 6 df to model the lag-response association. There were two outbreaks of Bluetongue Virus Serotype 8 in Belgium within the study period: from August to December 2006 and from July to December 2007.[34] Because the spread of Bluetongue in Belgium has been found to be associated with weather conditions,[35] we examined whether our results were robust to the exclusion of these epidemics from the analyses.

All analyses were performed using R project for statistical computing (version 3.1.2) using the "dlnm" package.

Results

Data description

Table 1 shows summary statistics for daily mortality, weather variables and air pollutants. There were 87,108 dairy cow deaths in Belgium between 2006 and 2009, with an average of 55 cases per day in the warm season and 65 cases per day in the cold season. The average daily mean temperature was 15.9°C in the warm season and 6.5°C in the cold season. Average relative humidity was 70.2% in the warm season and 81.0% in the cold season. The average concentrations of O_3, PM_{10} and NO_2 in the warm season were 80.4 µg/m^3, 24.6 µg/m^3 and 14.7 µg/m^3 respectively, whereas the corresponding concentrations in the cold season were 47.7 µg/m^3, 27.6 µg/m^3 and 21.5 µg/m^3 respectively.

Table 1. Summary statistics of daily cow mortality, weather conditions, and air pollution levels in Belgium, 2006-2009

			Percentiles				
Season	**Variable**	**Mean ± SD**	**0th**	**25th**	**50th**	**75th**	**100th**
Warm	Death	54.6 ± 22.2	12.0	38.0	53.0	68.5	145.0
(n=39,979)	Mean temperature (°C)	15.9 ± 4.0	4.1	13.5	16.1	18.5	28.6
	Humidity (%)	70.2 ± 10.9	32.1	64.1	70.7	78.1	94.5
	O_3 (µg/m^3)	80.4 ± 24.2	26.4	63.7	76.7	93.6	197.7
	PM_{10} (µg/m^3)	24.6 ± 10.8	8.9	16.7	21.7	29.8	83.1
	NO_2 (µg/m^3)	14.7 ± 6.0	3.2	10.2	13.8	18.5	35.8
Cold	Death	64.7 ± 25.8	1.0	45.0	65.0	83.0	162.0
(n=47,129)	Mean temperature (°C)	6.5 ± 4.5	-8.2	3.3	6.8	9.5	18.7
	Humidity (%)	81.0 ± 8.7	40.0	77.3	81.9	86.7	99.8
	O_3 (µg/m^3)	47.7 ± 18.0	9.4	34.7	48.8	60.7	98.6
	PM_{10} (µg/m^3)	27.6 ± 15.6	5.7	16.6	23.3	33.9	97.2
	NO_2 (µg/m^3)	21.5 ± 9.8	4.4	13.9	20.9	26.7	62.7

Abbreviations: SD, standard deviation; O_3, ozone; PM_{10}, particulate matter with diameter less than 10 µg/m^3; NO_2, nitrogen dioxide

Pearson correlation coefficients (r) between meteorological variables and air pollutants are presented in Table 2. The correlation between humidity and mean temperature was -0.34 in the warm season and -0.06 in the cold season. O_3 was positively correlated with mean temperature in both seasons (r=0.46 in the warm season and r=0.32 in the cold season). PM_{10} and NO_2 were negatively correlated with mean temperature in the cold season (r=-0.37 and r=-0.53 respectively), whereas corresponding correlations in the warm season were relatively low (r=0.12 and r=-0.06 respectively).

Table 2. Pearson correlation coefficients between weather conditions and air pollutants in Belgium, 2006–2009

Season	**Variable**	Temperature	Humidity	O_3	PM_{10}	NO_2
Warm	Temperature	1				
(n=39,979)	Humidity	-0.34*	1			
	O_3	0.46*	-0.71*	1		
	PM_{10}	0.12*	-0.26*	0.43*	1	
	NO_2	-0.06	-0.28*	0.29*	0.74*	1
Cold	Temperature	1				
(n=47,129)	Humidity	-0.06	1			
	O_3	0.32*	-0.40*	1		
	PM_{10}	-0.37*	-0.09*	-0.50*	1	
	NO_2	-0.53*	-0.01	-0.64*	0.78*	1

Abbreviations: O_3, ozone; PM_{10}, particulate matter with diameter less than 10 μg/m^3; NO_2, nitrogen dioxide
*P<0.05

DLNM analyses

The distributed non-linear lag surface obtained by the natural cubic spline–natural cubic spline DLNM revealed a nonlinear relationship between temperature and dairy cow mortality in the warm season, with higher mortality risk at high and low temperatures (Figure 1A). The three-dimensional plot for the cold season (Figure 1B) showed a similar shape, but the magnitude of effects was much smaller. Figure 2A presents the U-shaped dose-response curve for the overall effect of mean temperature on mortality over 25 days in the warm season, whereas the corresponding curve for the cold season (Figure 2B) was relatively flat. The heat and cold thresholds minimizing the residual deviance of the double threshold–natural cubic spline model were 16.8°C and 13.9°C in the warm season and 9.3°C and 5.9°C in the cold season. According to Q-AIC, the double threshold–natural

cubic spline DLNM fit the data better than the natural cubic spline–natural cubic spline DLNM in both seasons.

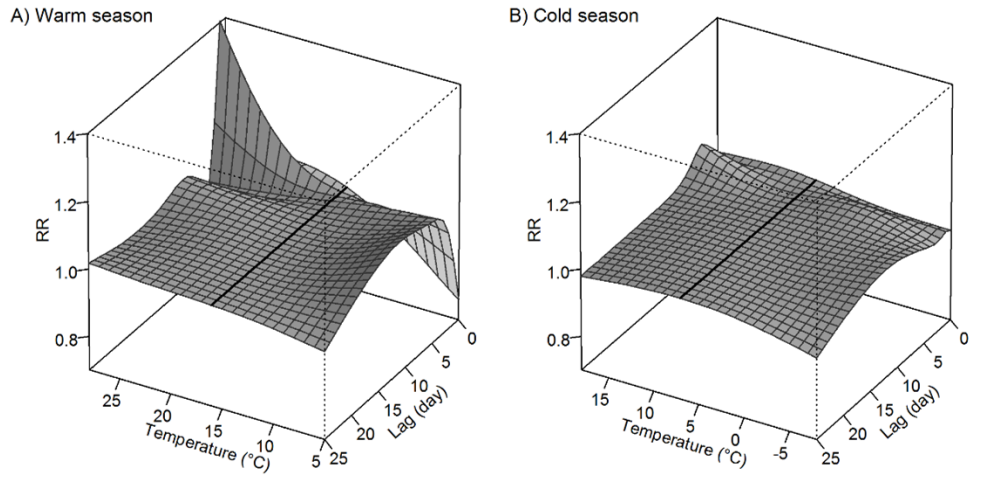

Figure 1. Relative risks (RR) of dairy cow mortality by mean temperature (°C) in the warm season (A) and in the cold season (B), using a natural cubic spline–natural cubic spline DLNM with 4 df for temperature and 6 df for lag. Reference values were median temperatures (16.1°C in the warm season and 6.8°C in the cold season).

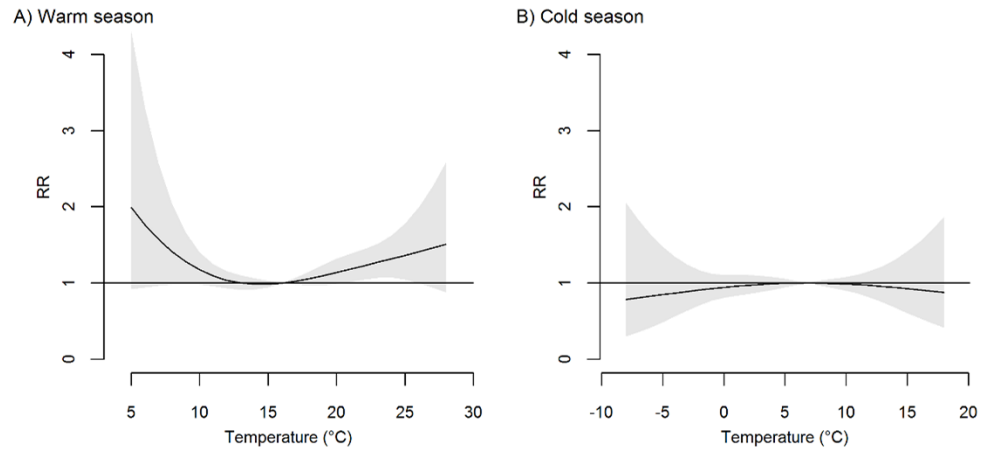

Figure 2. The estimated overall effect of mean temperature over 25 days on dairy cow mortality in the warm season (A) and in the cold season (B), using a natural cubic spline–natural cubic spline DLNM with 4 df for temperature and 6 df for lag. The black lines are the mean relative risks (RR) and the grey regions are 95% CIs.

Figure 3 presents the effects of a 1°C increase in mean temperature above the heat threshold (left panel) and a 1°C decrease in mean temperature below the cold threshold (right panel) along the lags. The heat effect in the warm season was acute, with the strongest and statistically significant effect on the day after the exposure (lag 1), and was followed by a deficit in mortality (mortality displacement) three to five days after the exposure. The effect of cold in the warm season was delayed by a few days and persisted for about 18 days. No significant lag-specific effects were observed in the cold season.

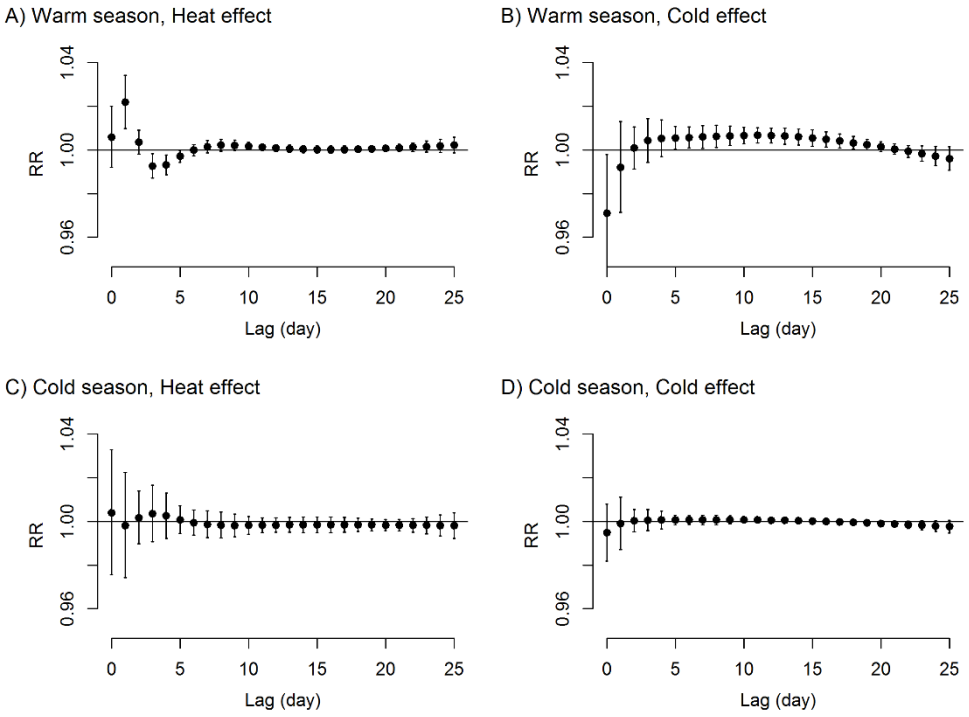

Figure 3. Lag-specific relative risks (RR, with 95% CIs) of dairy cow mortality for a 1°C increase in mean temperature above the heat threshold (A, C) and for a 1°C decrease in mean temperature below the cold threshold (B, D), in the warm season (A, B) and in the cold season (C, D), estimated using a double threshold–natural cubic spline DLNM with 6 df for lag. The heat and cold thresholds were 16.8°C and 13.9°C in the warm season and 9.3°C and 5.9°C in the cold season.

Over a short period of lag 0–2 days in the warm season, a 1°C increase in mean temperature above the heat threshold (16.8°C) was associated with a 3.16% (95% CI 1.64 to 4.71) increase in dairy cow deaths (Table 3). Incorporating the harvesting effect, the heat effect remained significant: over lag 0–10 days, the estimated increase in mortality for a 1°C increase in mean temperature above the heat threshold was 2.17% (95% CI 0.11 to 4.28). The overall cold effect during the warm season was higher than the heat effect: the estimated 26-day (lag 0–25) increase in mortality for a 1°C decrease in mean temperature below the cold threshold (13.9°C) was 4.90% (95% CI 0.40 to 9.60). Cumulative effects in the cold season were not significant.

Table 3. The cumulative heat and cold effects of mean temperature on dairy cattle mortality along the lag days, using a double threshold–natural cubic spline DLNM with 6 df for lag

		Percent increase in mortality (95% CI)	
Effect	**Lag (day)**	**Warm season**	**Cold season**
Heat†	0-1	2.80 (1.39 to 4.22)*	0.20 (-2.63 to 3.11)
	0-2	3.16 (1.64 to 4.71)*	0.37 (-2.77 to 3.61)
	0-10	2.17 (0.11 to 4.28)*	0.34 (-4.71 to 5.67)
	0-20	2.69 (0.16 to 5.28)*	-1.24 (-7.47 to 5.39)
	0-25	3.50 (0.54 to 6.55)*	-2.13 (-8.46 to 4.63)
Cold‡	0-1	-3.67 (-5.92 to -1.36)*	-0.62 (-1.63 to 0.41)
	0-2	-3.58 (-5.94 to -1.16)*	-0.59 (-1.66 to 0.50)
	0-10	0.96 (-2.60 to 4.65)	-0.07 (-1.51 to 1.40)
	0-20	5.81 (1.06 to 10.79)*	-0.07 (-2.24 to 2.14)
	0-25	4.90 (0.40 to 9.60)*	-0.96 (-3.68 to 1.83)

*P<0.05

†The percent increase in mortality for a 1°C increase in mean temperature above the heat thresholds (16.8°C in the warm season, 9.3°C in the cold season)

‡The percent increase in mortality for a 1°C decrease in mean temperature below the cold thresholds (13.9°C in the warm season, 5.9°C in the cold season)

Sensitivity analyses

Considering the acute (lag 0–2) heat effect in the warm season, results of the different sensitivity analyses were fairly consistent with those from the main analysis (supplementary Table S1): estimates from the unconstrained model and from models adjusting for O_3 or PM_{10} were lower, whereas the estimate from the model adjusting for NO_2 was higher. Adjustment for O_3 had the largest impact and significance of the heat effect at lag 0–10 (and later lags) was lost. The estimate of the overall (lag 0–25) cold effect in the warm season decreased slightly after

adjustment for PM_{10} or NO_2, thereby losing significance. However, the exclusion of Bluetongue epidemics from the study period resulted in a considerable increase in effect estimates (supplementary Table S2) and the overall cold effect remained significant even after adjustment for PM_{10} or NO_2.

Discussion

During the warm half of the year (April-September), high as well as low temperatures were associated with an increased risk of mortality among dairy cows in Belgium. The estimated heat and cold thresholds were 16.8°C and 13.9°C respectively. The effect of heat was acute and was followed by a deficit in mortality three to five days after the exposure, whereas the effect of cold was delayed and persisted for more than 2 weeks. We did not observe significant temperature effects during the cold season.

Heat-related increases in dairy cattle mortality were also found in some previous studies.[10-15] Most of them only focused on extreme heat episodes[10-12] and only few have investigated daily[14 15] or monthly[13] variations in mortality associated with temperature. Although comparisons are hampered by the use of different temperature indicators, our estimated heat threshold of 16.8°C is considerably lower than the heat thresholds reported for the warmer climates in California[13] and some regions in Italy.[14 15] However, our threshold is very close to the temperature-humidity index (THI) threshold of 16.7°C for Cuneo,[14] which has milder summer temperatures compared to the other Italian regions studied. The estimated increase in dairy cattle mortality for a 1°C increase in THI above the threshold at lag 0–2 for Cuneo was 2.7% (95% CI 1.1, 4.4),[14] which is similar to our estimate of 3.16% (95% CI 1.64 to 4.71). The estimates for the warmer climates of Brescia and Rome were 5.7% (95% CI 4.0–7.4%) and 7.5% (95% CI 4.6–10.6%) respectively.[14] The regional differences in estimated heat thresholds indicate some adaptation to local climatic conditions. This is supported by studies showing differences in hair coat characteristics between Holstein cows bred in temperate regions and cows bred in tropical and subtropical zones.[36]

When feed is converted by the animal's metabolism for the production of milk, eggs, meat, off-spring etc., heat is produced as a by-product. Lactating dairy cows create a large quantity of metabolic heat, making them much more vulnerable to

heat than to cold stress.[16 17] Consequently, the impact of cold on the performance and health of dairy cattle has received only limited research attention. Based on monthly averages, Stull *et al.*[13] reported a U-shaped association between temperature and dairy cow deaths. Another study focused on same-day exposures and did not find an effect of cold on dairy cattle mortality.[15] Our findings suggest that the association between cold and mortality is likely to be underestimated or even missed when using short lags. Despite the expected cold-tolerance of cows, we observed cold-related increases in mortality in the warm season, with the overall cold effect even being higher than the overall heat effect, whereas we did not find evidence for an association between temperature and mortality during the cold season. This might indicate that, being outdoors, free-ranging cows mainly suffer from the indirect effects of cold, such as precipitation and wind speed. Heavy rain my penetrate the fur of an animal and decrease its insulation value and a strong wind leads to additional excessive cooling. On the other hand, a multi-country study on human mortality also found residual cold effects in the four warmest months of the year (under review, personal communication).

The thermal comfort zone for lactating dairy cows is estimated to lie between −0.5 and 20°C,[37] which is cooler than the optimal temperature range for humans. However, biochemical and physiological changes in response to thermal stress are expected to be similar for both species. Similar to findings for human mortality,[38-44] we found acute effects of high temperatures and more delayed and prolonged effects of low temperatures. Also consistent with human studies is the observation that heat effects are (partly) compensated by a deficit in mortality at later lags, whereas harvesting effects are not found for cold effects.[38 40 42 43] A comparison of our results with those from Italian cattle further suggests that heat-related mortality among dairy cattle might relate to local climate in the same way as observed among humans, i.e. higher heat thresholds in warmer compared with colder climates.[43-45] In a study on human mortality in 15 European cities, the meta-analytic estimate of the threshold for daily maximum apparent temperature was 29.4°C for Mediterranean cities and 23.3°C for north-continental cities,[43] and the estimated changes in mortality for a 1°C increase above these thresholds were 3.12% (95% CI 0.60 to 5.72) and 1.84% (95% CI 0.06 to 3.64) respectively.[43] The heat effect estimates for human appear to be slightly smaller than the corresponding estimates for Mediterranean cattle[14] and north-continental cattle

(our study), which is likely to be related to the lower thermal comfort zone[46] combined with higher exposure levels in free-ranging animals.

A large part of temperature-related human deaths is caused by respiratory and cardiovascular conditions. The studies on human mortality in 15 European cities suggest that the effect of temperature on respiratory deaths is nearly twice as large as the effect on cardiovascular deaths, both for heat effects[43] and for cold effects.[38] Although there is large uncertainty about exact causes of death in dairy cattle,[47 48] cows are known to be susceptible for pulmonary diseases because of anatomical and physiological differences with other mammals.[49] Therefore we expect that a considerable part of temperature-related mortality among cattle might be due to respiratory conditions.

Results of sensitivity analyses suggested that the association between temperature and dairy cattle mortality might be confounded by air pollution, which has also been observed in studies on human mortality.[43 50 51] Adjustment for O_3 resulted in a reduction in the estimated heat effect, whereas its impact on the cold effect was negligible, and the other way around for PM_{10} and NO_2. This is not surprising given the strong positive correlation between temperature and O_3, whereas highest PM_{10} and NO_2 concentrations are observed on cold days.

Our study has some limitations. Meteorological variables and air pollution concentrations were derived from outdoor monitoring stations, so that the individual exposure levels may have differed substantially from the estimated exposure. However, issues such as confounding and exposure misclassification are expected to be less problematic in our animal population than in human studies. As dairy cattle have limited population variability in lifestyle and dietary habits, confounding effects of such factors is greatly reduced. Moreover, the restricted daily mobility, the lower frequency of migration, and the partial outdoor housing of dairy cattle contribute to the likelihood that exposure assessment can be conducted more accurately than in human populations. On the other hand, the primary effect of temperature on free-ranging animals might be strongly altered by wind, precipitation, humidity, and solar radiation.[52] Moreover, observed results might be influenced by the effects that weather may exert on quantity and quality of pasture and water, or on survival and growth of infectious agents.[53]

Nevertheless, Stull *et al.*[13] suggested that precipitation had little or no effect on dairy cattle mortality that was independent of temperature.

Europe experienced a record-breaking heat wave in August 2003, with an estimated death toll of around 70,000 people. This has initiated efforts to implement heat plans and real-time monitoring of human mortality at national[54-56] and at European level.[57 58] Monitoring of all-cause deaths provides a major source of timely data on progression and impact of health threats, which are crucial to guide health service response and public health decision-making. Not only the expected increase in extreme weather events due to climate change has highlighted the need for early warning systems, but also other public health threats such as air pollution and the potential of infectious and non-infectious diseases to (re-)emerge. While the lack of timely data is often a hampering factor for mortality surveillance, the mortality monitoring system in Belgium (Be-MOMO) provides a relatively rapid warning of mortality peaks, *i.e.* within one to two weeks after the event.[55] Similar systems could be implemented for the monitoring of livestock mortality. Dairy cattle have the unique property that they are subject to a stringent individual-level registration procedure, which is mandatory in many countries, resulting in the availability of timely mortality data. In Belgium, the death of a cow has to be reported to Sanitel within seven days. A routine monitoring of dairy cattle mortality might be useful for a rapid detection of health risks among animals, human or both.

Conclusion

This study showed significant increases in dairy cattle mortality associated with low and high ambient temperatures in the warm season. Dairy cows are expected to have similar biological responses to environmental exposures as humans, but they have limited geographic mobility, and may be exposed to higher concentrations than human. Therefore, they may be sensitive indicators of environmental hazards and provide an early warning system for public health intervention.

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Supplementary material

Table S1. The cumulative heat and cold effects of mean temperature on dairy cattle mortality along the lag days in the warm season, estimates from the main model and different sensitivity models

		Percent increase in mortality (95% CI)									
Effect	**Lag (day)**	**Main§**		**Unconstrained¶**		**O_3 adjusted§#**		**PM_{10} adjusted§#**		**NO_2 adjusted§#**	
Heat†	0-1	2.80	(1.39 to 4.22)*	4.42	(2.64 to 6.23)*	1.60	(-0.12 to 3.35)	2.55	(1.08 to 4.05)*	2.55	(1.12 to 4.00)*
	0-2	3.16	(1.64 to 4.71)*	2.39	(0.36 to 4.46)*	2.06	(0.15 to 4.00)*	2.99	(1.39 to 4.62)*	3.41	(1.85 to 5.00)*
	0-10	2.17	(0.11 to 4.28)*	2.13	(-0.39 to 4.71)	-0.08	(-3.37 to 3.32)	2.36	(0.06 to 4.72)*	2.69	(0.54 to 4.88)*
	0-20	2.69	(0.16 to 5.28)*	2.45	(-0.44 to 5.42)	1.16	(-3.39 to 5.92)	2.21	(-0.68 to 5.18)	2.80	(0.21 to 5.45)*
	0-25	3.50	(0.54 to 6.55)*	2.78	(-0.24 to 5.88)	2.17	(-3.09 to 7.70)	2.84	(-0.47 to 6.26)	3.50	(0.53 to 6.57)*
Cold‡	0-1	-3.67	(-5.92 to -1.36)*	-2.27	(-5.37 to 0.92)	-3.80	(-6.03 to -1.51)*	-3.35	(-5.73 to -0.91)*	-2.93	(-5.26 to -0.55)*
	0-2	-3.58	(-5.94 to -1.16)*	-5.26	(-8.77 to -1.61)*	-3.65	(-6.00 to -1.24)*	-3.22	(-5.72 to -0.65)*	-2.93	(-5.37 to -0.44)*
	0-10	0.96	(-2.60 to 4.65)	-0.10	(-4.90 to 4.93)	1.27	(-2.34 to 5.00)	0.07	(-3.64 to 3.93)	0.27	(-3.32 to 3.99)
	0-20	5.81	(1.06 to 10.79)*	4.69	(-0.48 to 10.13)	5.84	(1.01 to 10.89)*	5.06	(0.02 to 10.34)*	5.40	(0.48 to 10.55)*
	0-25	4.90	(0.40 to 9.60)*	4.86	(0.22 to 9.72)*	4.77	(0.16 to 9.59)*	4.09	(-0.51 to 8.90)	4.29	(-0.26 to 9.05)

Abbreviations: O_3, ozone; PM_{10}, particulate matter with diameter less than 10 μg/m^3; NO_2, nitrogen dioxide

*P<0.05

†The percent increase in mortality for a 1°C increase in mean temperature above the heat threshold (16.8°C)

‡The percent increase in mortality for a 1°C decrease in mean temperature below the cold threshold (13.9°C)

§Double threshold–natural cubic spline DLNM with 6 df for lag

¶Double threshold–unconstrained DLNM

#Additionally adjusted for O_3, PM_{10}, or NO_2 by means of a linear–natural cubic spline crossbasis with a maximum lag of 25 (1 df for the air pollutant and 6 df for lag)

Table S2. The cumulative heat and cold effects of mean temperature on dairy cattle mortality along the lag days in the warm season after exclusion of the Bluetongue epidemics (August–December 2006 and July–December 2007), estimates from the main model and different sensitivity models

		Percent increase in mortality (95% CI)									
Effect	**Lag (day)**	**Main§**		**Unconstrained¶**		**O_3 adjusted§#**		**PM_{10} adjusted§#**		**NO_2 adjusted§#**	
Heat†	0-1	3.50	(1.83 to 5.19)*	5.50	(3.46 to 7.58)*	2.00	(-0.10 to 4.14)	3.09	(1.33 to 4.87)*	2.90	(1.16 to 4.66)*
	0-2	3.95	(2.15 to 5.77)*	2.92	(0.60 to 5.29)*	2.37	(0.06 to 4.73)*	3.78	(1.89 to 5.71)*	3.92	(2.05 to 5.83)*
	0-10	3.08	(0.65 to 5.57)*	3.13	(0.14 to 6.20)*	-0.23	(-3.87 to 3.56)	3.26	(0.65 to 5.94)*	3.32	(0.85 to 5.84)*
	0-20	3.93	(0.46 to 7.52)*	3.18	(-0.64 to 7.15)	1.82	(-3.19 to 7.10)	3.17	(-0.53 to 7.00)	3.37	(-0.14 to 7.01)
	0-25	5.71	(1.54 to 10.04)*	4.88	(0.66 to 9.28)*	4.65	(-1.25 to 10.91)	4.95	(0.57 to 9.52)*	5.07	(0.92 to 9.39)*
Cold‡	0-1	-3.48	(-5.87 to -1.04)*	-2.07	(-5.40 to 1.39)	-3.71	(-6.07 to -1.29)*	-3.12	(-5.64 to -0.54)*	-2.92	(-5.39 to -0.38)*
	0-2	-3.41	(-5.89 to -0.85)*	-6.21	(-9.94 to -2.33)*	-3.51	(-5.98 to -0.97)*	-3.02	(-5.66 to -0.30)*	-2.80	(-5.37 to -0.15)*
	0-10	1.85	(-1.93 to 5.78)	0.33	(-4.81 to 5.74)	2.70	(-1.19 to 6.74)	0.75	(-3.22 to 4.87)	0.88	(-2.96 to 4.87)
	0-20	6.97	(1.89 to 12.30)*	5.85	(0.37 to 11.64)*	7.59	(2.37 to 13.07)*	6.69	(1.25 to 12.43)*	6.84	(1.53 to 12.42)*
	0-25	6.07	(1.26 to 11.12)*	6.01	(1.04 to 11.23)*	6.25	(1.24 to 11.50)*	5.23	(0.32 to 10.37)*	5.27	(0.38 to 10.39)*

Abbreviations: O_3, ozone; PM_{10}, particulate matter with diameter less than 10 $\mu g/m^3$; NO_2, nitrogen dioxide

*P<0.05

†The percent increase in mortality for a 1°C increase in mean temperature above the heat threshold (16.8°C)

‡The percent increase in mortality for a 1°C decrease in mean temperature below the cold threshold (13.9°C)

§Double threshold–natural cubic spline DLNM with 6 df for lag

¶Double threshold–unconstrained DLNM

#Additionally adjusted for O_3, PM_{10}, or NO_2 by means of a linear–natural cubic spline crossbasis with a maximum lag of 25 (1 df for the air pollutant and 6 df for lag)

Chapter 8

General discussion

In this doctoral dissertation population-based registries were used to assess the impact of important environmental risk factors on population health and to evaluate preventive public health measures. We used a life-course approach by including both studies on newborns and studies focusing on the older segment of the population, thereby focusing on health outcomes for which certain research gaps have recently been identified. We verified human epidemiological findings in registries of dairy cattle which might serve as a sentinel for human public health. We made use of the following data sources: the Flemish birth registry, the Flemish registry of cause-specific mortality data and the Belgian registry of farm animals.

The novelties of this dissertation include:

- The evaluation of a stepwise implementation of smoke-free legislation by studying potential subsequent changes in adverse health outcomes in early and later life
- The investigation of ambient temperature as a new environmental risk factor for preterm birth
- The exploration of the shape of the association between maternal PM_{10} exposure and fetal growth
- The use of dairy cattle as a sentinel for the effects of environmental exposures on all-cause mortality
- The application of a relatively new statistical method (distributed lag non-linear models) to study the temporal pattern of exposure-response associations

1. The use of population-based registries in epidemiological research

As reviewed recently,[1] a substantial portion of the annual global investment in biomedical research does not lead to worthwhile achievements, partly because experimental studies done to improve the understanding of basic mechanisms in disease have little relevance for human health or fail to be translated from bench to clinical application or epidemiological observations. For instance, acute inflammatory stresses from different etiologies resulted in highly similar genomic responses in mouse models and humans, but the responses in mice correlated

poorly with the human conditions.[2] These findings support the value of population-based studies.

Nevertheless, observational epidemiological studies cannot prove causation as they tend to be more prone to bias than randomized experimental studies. Bias due to confounding occurs when the observed association is due, totally or in part, to another factor than the exposure (or intervention) under investigation. Information bias (measurement error) can arise when the outcome, the exposure (or intervention), or potential confounders are inaccurately reported or measured. A third type of systematic error is selection bias which occurs when study entry or exit is related to the outcome or to the exposure (or intervention). Each of these type of errors are discussed in relation to characteristics of the registry data and statistical methods used.

A first source of bias, common to many epidemiological studies on air pollution and temperature, is the use of outdoor monitoring stations to estimate individual exposures, which results in exposure misclassification (information bias). Actual individual exposures might be quite different from outdoor air pollution concentrations and/or temperatures due to factors such as mobility, clothing, time spent indoor, indoor heating, air conditioning and ventilation. However, as discussed in chapter 5, exposure misclassification is likely to be unrelated to the health outcome (non-differential misclassification), resulting in an underestimation of effect estimates.

All research questions in this dissertation were addressed by "secondary" registry data. Secondary data sources are comprised of data which have been collected for purposes other than a specific research question, such as surveillance, control, management, administration or evaluation. Population-level registries have the major advantage that they have a (nearly) complete coverage of the population, giving large and representative samples which are less likely to suffer from recall or non-response bias (selection bias) than primary data sources. Another advantage of secondary registry data is that they already exist, thereby significantly reducing the time and cost spent on the collection of data.

The disadvantages of secondary data are related to the fact that the investigator has little or no control over what data have been collected, and how. Specific information may not have been collected or may not be available to the secondary

researcher for confidentiality reasons. None of the registries used in this dissertation provided information on the exact home addresses of the cases, implying that linkage with air pollution data could only be done at the level of the municipality of residence and local variations in exposure are likely to be missed (exposure misclassification).

Common to many population-based registers, we also did not have information on potential confounders such as diet and smoking. The use of statistical methods such as Poisson regression (where exposure is defined at the group level) and case-crossover analyses (where each subject serves as his/her own control) avoids confounding by individual risk factors that do not change over short durations of time. In logistic (chapters 2 and 5) and linear (chapter 5) regression analyses, models were adjusted for the relevant individual variables available. Time-varying factors such as long-term trends, seasonality and day of the week were controlled for by including appropriate variables in the model (Poisson and Logistic models) or by selecting control periods which are close in time to the case period and by additionally matching on day of the week (case-crossover analysis).

Additional information might be gathered by merging the registry with other data sources, although a 100% match can often not be obtained. For instance, socio-demographic variables such as parental education and national origin of the mother are missing in the medical birth certificates of the Study Centre for Perinatal Epidemiology (SPE), but are obtained through linkage with official birth declarations. However, the additional variables remained missing for a considerable number of individuals and the linkage was only available for a specific time period (1999-2009). Although statistical adjustment for these variables (as done in the analysis on particulate matter and fetal growth, chapter 5) might introduce selection bias, sensitivity analyses showed that the exclusion of individuals with missing covariate information had little effect.

When using secondary registries, the investigator has no control over the quality or validity of the data, which might result in information bias. However, for large-scale population-level registries data collection is often guided by expertise and professionalism that may not be available to individual researchers or small research projects. Administrative systems that register deaths are relatively complete and robust, and causes of death are coded in an internationally

comparable manner by using a specific diagnostic system (the WHO International Classification of Diseases). Still most evaluations of cause of death data demonstrate diagnostic difficulties and errors in coding and classifications.[3] However, a study from the Netherlands showed that the reliability of cause of death statistics was high (>90%) for major causes of death such as acute myocardial infarction and cancers, and low (<70%) for chronic diseases such as diabetes and renal insufficiency.[4] Also the quality of perinatal data is expected to be high as the SPE takes the following measures to obtain an optimal registration of births:[5 6] 1) guidelines on how to complete the obstetric and perinatal file are sent to the maternity units on an annual basis; 2) the quality of data is checked for internal inconsistencies, accuracy and completeness by using an error detection program; 3) maternity units are contacted in case of inconsistent or missing data.

Even in case of misclassification of acute myocardial infarction deaths, there is no reason to suspect that there was a systematic error as a result of a change in the percentage of misclassified cases after implementation of the smoking bans. Likewise, potential changes in the estimation of gestational age, in the measurement of birth weight, or in the registry of dairy cow deaths are not expected to be related to the implementation of smoking policies, nor to short-term variations in temperature or air pollution.

Still, residual confounding may always exist in epidemiological studies. It might be that unmeasured confounders were responsible for the observed reductions in preterm births and/or acute myocardial infarction mortality. It is, however, unlikely that our observations could be explained by abrupt changes in therapeutic strategies coinciding with the smoking bans. There were no substantial changes in the prescription of Atosiban (used to halt premature labor) and in cervical cerclage treatment (used in case of cervical incompetence) during the study period.

Compared to human studies, studies on dairy cattle are expected to be less subject to issues such as confounding and exposure misclassification because dairy cows have limited population variability in lifestyle and dietary habits, limited geographical mobility and (partial) outdoor housing.

2. Discussion of study findings

2.1 Health impact of smoke-free legislation

2.1.1 Perinatal and child health

Although it is well accepted that smoking during pregnancy increases the risk for adverse birth outcomes, evidence for early life health benefits related to smoking policies was very limited at the time of our study.[7-11] Two studies had investigated the effect of smoking bans on asthma events among children. The first study (USA, Lexington-Fayette county, KY)[7] reported an immediate drop in pediatric asthma emergency department visits of 18% and the other study (UK, Scotland)[11] found a more gradual decrease in asthma admissions of 20% per year. The same authors examined the impact of the Scottish smoking ban on pregnancy outcomes.[9] They found immediate reductions in the risk of small-for-gestational-age (-5%), low birth weight (-10%), and preterm birth (-12%), but the decrease in preterm births was followed by a significant gradual increase of 4% per year. In Ohio (USA), smoke-free legislation was found to be associated with an annual decrease in low birth weight of 1%, but no significant change in the preterm birth rate was observed.[8] In Dublin, the decrease in the risk of preterm birth after the implementation of a smoking ban was estimated at 25%, but the risk of low birth weight increased by 43%.[10] However, the analysis was based on a very short study period (one year before and after the ban) and did not take into account potential time trends.

The stepwise implementation of smoke-free legislation in Belgium gave us the unique opportunity to study potential successive changes in the risk of passive smoking related health outcomes. Based on 448,520 non-induced singleton live births in Flanders between 2002 and 2011, we found an immediate change in the risk of preterm birth of -3.13% (95% CI -4.37 to -1.87) on 1 January 2007 (smoking ban in restaurants), and a gradual annual change of -2.65% (95% CI -5.11 to -0.13) since 1 January 2010 (smoking ban in bars serving food).[12] To put this in perspective, this corresponds to a reduction of 6 preterm births per 1000 deliveries. One of the strengths of our study is the adjustment for underlying trends and seasonal effects, for different individual characteristics (such as socioeconomic status and national origin of the mother) and other risk factors (such as air pollution and influenza epidemics). Given the growing evidence that

a reduction in gestational age is associated with morbidity and mortality in children[13 14] as well as in adults,[15] even a small reduction in preterm births is a major benefit for public health.

Since then, a number of other studies investigated the effect of smoke-free legislation on perinatal and child health. To summarize the existing evidence worldwide, we participated in an international collaboration with colleagues from Maastricht, Edinburgh, and Harvard universities. Results of this study were published in The Lancet.[16] The meta-analysis was based on 11 eligible studies (published between 2008 and 2013), including more than 2.5 million births and 247,168 asthma exacerbations. Five of the studies involved local smoking bans in North America and the other six involved national bans in Europe. All of them used interrupted time-series regression models. Forests plots summarizing the results of the meta-analysis are presented in Figure 1. Smoke-free legislation was found to be associated with a 10.4% (95% CI 18.8 to 2.0) reduction in preterm birth (based on four studies) and a 10.1% (95% CI 15.2 to 5.0) reduction in hospital attendances for asthma (based on three studies). The reduction in low birth weight (1.7%, 95% CI -5.1 to 1.6, based on six studies) was not significant. These results indicate that the findings for Flanders are consistent with those from other countries.

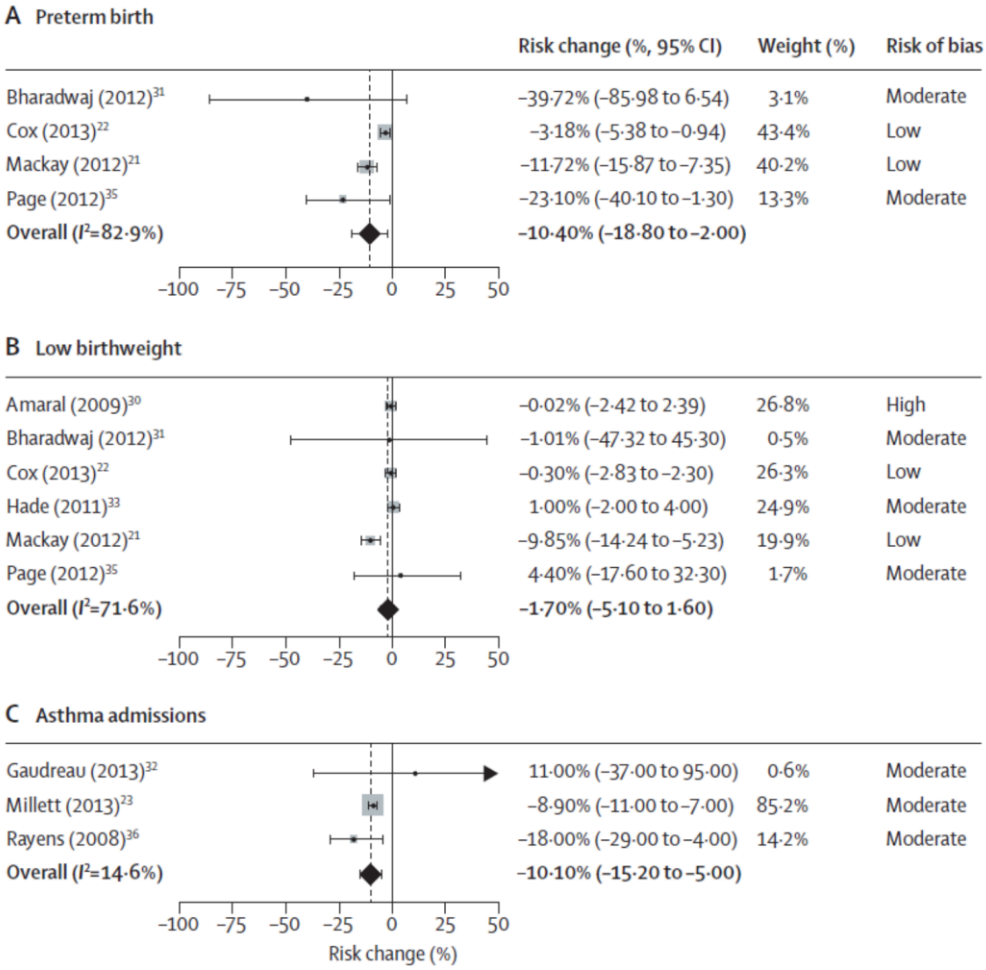

Figure 1. Meta-analytic estimates of step changes in (A) preterm birth, (B) low birthweight, and (C) asthma admissions after implementation of smoke-free legislation (Been JV, Nurmatov UB, Cox B, *et al*. Lancet 2014)[16]

2.1.2 Acute myocardial infarction mortality

We also investigated the potential public health benefit of smoke-free legislation in an older segment of the population by focusing on deaths due to acute myocardial infarction (AMI).[17] Although numerous studies have demonstrated reductions in cardiovascular hospital admissions related to smoking policies, evidence for similar effects on cardiovascular mortality was limited. Using data from all fatal AMIs in Flanders between 2000 and 2009 (n=38,992), we observed

an immediate decrease in the risk of AMI after the implementation of the smoking ban at the workplace (January 2006). The effect was largest for women younger than 60 years. Among elderly men, we found an additional gradual effect of the smoking ban in restaurants since 1 January 2007. The magnitude of the overall reduction in AMI mortality rates associated with the 2006 legislation found in our study (-15.3% among people aged under 60 years and -8.3% among people aged 60 years or older) is comparable with the 10–17% decrease in AMI hospital admissions rate estimated by four meta-analyses.[18-21] The estimated effect for women younger than 60 years in our study, however, is considerably higher (-33.8%), which might be explained by a stronger decrease in exposure among women, as observed in Scotland,[22] or by a higher relative risk for AMI associated with smoking in women compared with men.[20] Our findings correspond to an estimated total of 1715 fatal AMIs averted from 2006 to 2009. Strengths of our study include the long study period and the adjustment for important factors such as (non-linear) long-term trends.

For both preterm birth and AMI mortality, we found reductions associated with different steps of the smoking ban policy. Hill proposed both internal and external consistency as one of the most important criteria of causality.[23] Although a single epidemiological study cannot prove causality, we believe that a strength of our studies is the internal consistency, as suggested by the observed subsequent reductions in adverse health outcomes.

2.2 Effect of temperature and air pollution on birth outcomes

Despite extensive research efforts, the factors that cause adverse birth outcomes are not well understood. For preterm birth for example, no clear cause can be isolated for more than 50% of cases.[24] Genetics, nutrition (such as vitamin deficiency and alcohol/caffeine intake), and maternal disease (such as diabetes and untreated infections) are known to affect fetal development. More recent research has focused on potential effects of environmental exposures including high ambient temperatures and chemicals such as polychlorinated biphenyls, pesticides, water contaminants, environmental tobacco smoke, and air pollution.[25]

2.2.1 Preterm birth and ambient temperature

Based on 6 studies, a review from 2011 concluded that evidence for short-term effects of temperature on preterm birth is still limited.[26] A similar review from 2014 included 7 studies, from which 4 had reported a significant association between temperature and preterm birth.[27] Both reviews concluded that inconsistencies between study results are likely to be related to the large variation in study designs, statistical approaches, confounder adjustments, exposure windows, and sample sizes. Moreover, most studies assumed a linear association between temperature and preterm birth, whereas J- or U-shaped associations have typically been reported in studies of temperature-related morbidity and mortality in adults. As pointed out by Strand *et al.*,[26] the use of a linear association when the effect is actually U-shaped will result in an underestimation of the association strength. Another issue, raised by Darrow *et al.*,[28] is the potential confounding by gestational age when aggregating fetuses at risk across different gestational ages, thereby ignoring the fact that the risk of preterm birth increases exponentially with higher gestational ages.

Taking into account these methodological issues and using data on 446,110 births, we investigated the association between temperature and preterm birth in the temperate climate of Flanders. We estimated that the 3-day (lag 0-2) cumulative risk of preterm birth in the warm season increased by 14.1% (95% CI 4.7 to 24.2) for an increase in minimum temperature from the 50th to the 95th percentile (12.8°C and 17.9°C respectively). We also observed a significant increase in preterm birth two days after exposure to low temperatures in the cold season, but cumulative 3-day effects were not significant. An overview of currently available studies on the association between temperature and preterm birth is given in Table 1. From the four studies conducted on populations in a colder climate (UK,[29] Germany,[30] Sweden,[31] our study), significant temperature effects were only found in our study and in the historical study population (1915-1929) from Uppsala (Sweden).[31] A comparison of study findings is hampered by the use of different modelled outcomes and different exposure windows, and even between studies using similar approaches there is considerable variation in effect estimates. Nevertheless, the growing evidence indicates that, in addition to the known association between maternal hyperthermia and birth defects,[32] heat might also be a risk factor for preterm birth. Therefore, prenatal care workers should

recommend pregnant women to protect themselves from high temperatures, e.g. by avoiding the use of hot tubs, sauna and electric blankets.

Table 1. Summary of existing studies on short-term effects of ambient temperature on preterm birth

Study	Country (region)	Study period	N births	Statistical method	Exposure (lag)	Findings
Lajinian (1997)[33]	USA (Brooklyn)	21/3/1993 – 20/03/1994	NR	Exact test for linear trend on preterm labor and preterm birth rates during 7 coldest and warmest days of winter and summer	Mean AT (lag 0–6)	Significant trend for preterm labor (rate from 1.23% to 3.00%) but not for preterm birth
Porter (1999)[34]	USA (Chicago)	01/06/1995 – 31/08/1995	11,792	Independent t-tests comparing mean GA between strata defined by confounder and temperature categories.	Max AT (lag 0 to lag 2)	No significant difference in mean GA between confounder* temperature strata
Lee (2008)[29]	UK (London)	1988 – 2000	482,568	Logistic time-series regression with the number of fetuses-at-risk as the denominator	Min T, Max T (lag 0, lag 0–1 to lag 0–6)	No significant association between preterm birth risk and T
Yackerson (2008)[35]	Israel (Negev)	1999	11,979	Unclear (Multivariate analysis, time series approach and Poisson regression)	Max T (lag 3)	Significant association between number of preterm births and T
Flouris (2009)[36]	Greece	1999 – 2003	516,874	Correlation with GA	Mean T (month of birth)	Significant correlation between GA and T (r=-0.21; P<0.001)
Basu (2010)[37]	USA (California)	1999 – 2006	58,681 (preterm)	Time-stratified case-crossover	Mean AT (lag 0–6)	8.6% (95% CI 6.0 to 11.3) increase in preterm birth risk for a 5.6°C increase in T
Dadvand (2011)[38]	Barcelona (Spain)	2001 – 2005	7,585	Two-stage analysis: linear regression with GA as dependent variable and as offset the predicted monthly trend in GA predicted in stage 1	Mean AT (lag 1)	5.3-day reduction in the average length of pregnancy (95% CI 0.05 to 10.1) for T above the 99th pct
Strand (2011)[39]	Brisbane (Australia)	2005 – 2009	101,870	Cox proportional hazards regression	Mean T (last 4 weeks)	2% increase in preterm birth risk for T increase from 21°C to 25°C
Wolf (2012)[30]	Brandenburg & Saxony (Germany)	2002 – 2010 & 2005 – 2009	128,604 & 162,913	Logistic time-series regression with the number of fetuses-at-risk (stratified by GA) as the denominator and corrected for GA (as factor)	Mean T (lag 0–6)	No significant association between preterm birth risk and T
Schifano (2013)[40]	Italy (Rome)	2001 – 2010	132,691	Poisson generalized additive model with daily number of pregnancies at risk as offset	Max AT (lag 0–2)	1.87% (95% CI 0.86 to 2.87) increase in preterm birth risk for a 1°C increase in T
Wang (2013)[41]	Brisbane (Australia)	2000 – 2010	50,848	Cox proportional hazards regression	Heat waves (last weeks)	13% (95% CI 3 to 24) to 100% (95% CI 37 to 191) increase in preterm birth risk depending on heat wave definition
Auger (2014)[42]	Montreal (Canada)	1981 – 2010	206,929	Cox proportional hazards regression	Max T (lag 0–6)	Association for term birth (4% increase in the risk of birth for T increase from 20°C to 35°C) but not for preterm birth

Table 1. (continued)

Study	Country (region)	Study period	N births	Statistical method	Exposure (lag)	Findings
Vicedo-Cabrera (2014)[43]	Valencia (Spain)	2006 – 2010	20,148	Quasi-Poisson generalized additive models with daily number of fetuses-at-risk as offset and corrected for GA seasonality (as smooth term)	Max AT (lag 2)	20% increase in preterm birth risk for T increase from 22.5 (50th pct) to 36.1°C (90th pct)
					Min T (lag 4 to lag 6)	5% increase in preterm birth risk for T increase from 11.7 (50th pct) to 22.3°C (90th pct)
Bruckner (2014)[31]	Uppsala (Sweden)	1915 – 1929	13,657	Cox proportional hazards regression	Mean T (lag 0–6)	40% (95% CI 29 to 49) increase in preterm birth risk for a 1°C decrease in T below the 25th pct and 52% (95% CI 22 to 90) increase for a 1°C increase in T above the 75th pct
Our study	Belgium (Flanders)	1998 – 2011	446,110	Quasi-Poisson models with daily number of fetuses-at-risk as offset and corrected for GA seasonality (as smooth term)	Min T (lag 0–3)	14.1% (95% CI 4.7 to 24.2) increase in preterm birth risk for T increase from 12.8°C (50th pct) to 17.9°C (95th pct)

Abbreviations: AT, apparent temperature; GA, gestational age; Max, maximum; Min, minimum; NR, not reported; pct, percentile; T, temperature

2.2.2 Fetal growth and air pollution

In 2011 a review summarized the evidence from 41 studies on the association between air pollutants and low birth weight, preterm birth and/or small-for-gestational-age.[44] The authors concluded that reported associations, and lack thereof, between individual air pollutants and birth outcomes differed across published studies. A meta-analyses from 2012, based on 61 studies, concluded that existing evidence is indicative of associations between CO, NO_2, PM and pregnancy outcomes, but recommended that variation in effects by exposure period and sources of heterogeneity between studies should be further explored.[45]

As pointed out in the review of Shah *et al.*,[44] exposure to pollution in the first trimester could affect implantation, while exposure in the third trimester could affect fetal growth. Moreover, most previous studies only considered exposures averaged over longer time windows (such as trimesters and entire pregnancy). Consistent low exposure levels associated with occasional high exposure will lead to an average exposure assessment of "moderate", despite the fact that the biological impact of consistent moderate exposure may be different from the former scenario.[44] Therefore, in addition to the investigation of average pregnancy and trimester exposures, we also examined shorter exposure windows during critical stages in early and late pregnancy. Whereas most previous studies have restricted their study population to term births, we also included preterm births in our analyses in order to examine potential effect modification by gestational age.

Using data on a total of 525,635 birth in Flanders, we found significant associations between *in utero* PM_{10} exposure and fetal growth among babies born after 31 weeks of gestation. For both outcomes studied (birth weight and small-for-gestational-age), effects of maternal PM_{10} exposure were considerably higher for neonates born moderately preterm (32-36 weeks) than for those born at term. The estimated reduction in birth weight for a 10 $\mu g/m^3$ increase in average PM_{10} during pregnancy was 39.0 g (95% CI 26.4 to 51.5) for moderately preterm births and 24.0 g (95% CI 20.9 to 27.2) for term births. The corresponding odds ratios for small-for-gestational-age were 1.19 (95% CI 1.07 to 1.32) and 1.09 (95% CI 1.06 to 1.12) respectively. It should be noted that the reduction in term birth weight estimated in our study (24 g per 10 $\mu g/m^3$ PM_{10}) is nearly threefold the corresponding estimates from large-scale meta-analytic[45] and multi-country[46 47] studies (around 8 g per 10 $\mu g/m^3$ PM_{10}). In addition to the finding of effect

modification, another relatively novel aspect of our study is the investigation of the shape of the association between PM_{10} and fetal growth. Segmented regression models showed significant departures from linearity, with stronger effects of PM_{10} at lower concentrations and a flattening out of the slope at higher concentrations.

2.3 Effect of temperature and air pollution on cattle mortality

Observational studies of spontaneous disease in animal populations can provide additional insights not available from laboratory-based studies of experimental animals.[48] An early example of the usefulness of sentinels to identify environmental hazards dates from the beginning of the 20st century, when canaries were used in coal mines to warn of high levels of carbon monoxide or other impurities in the air.[49] Much of the previous work has focused on cancers in pet animals, particularly dogs, as they share the environment and are exposed to many of the same agents as their human companions.[50] Yet the full potential of linking animal and human health data to provide warning of shared environmental risks has not been realized.[48]

We addressed the use of dairy cattle as models for epidemiologic studies of human mortality and ambient air pollution and temperature exposures. To the best of our knowledge, no studies exist on the association between air pollution and dairy cattle deaths, and only few studies have investigated the effect of temperature,[51-56] most of them only focusing on heat effects.[51-53 55] We used daily data on cattle mortality which are readily available from a national-level database for the registration and traceability of farm animals (Sanitel). We found a significantly increased mortality risk associated with exposure to air pollution and temperature extremes during the warm period of the year (April – September), but not during the cold season (October – March). Estimated short-term increases in mortality for a 10 $\mu g/m^3$ increase in O_3 (lag 0-1), PM_{10} (lag 0) and NO_2 (lag 0) were 1.3% (95% CI 0.3 to 2.2), 1.2% (95% CI -0.3 to 2.8) and 9.4% (95% CI 6.4 to 12.4) respectively. Incorporating harvesting and delayed effects, the 26-day (lag 0–25) cumulative risks of mortality were 3.6% (95% CI 0.4 to 6.9), 5.1% (95% CI 0.8 to 9.5) and 2.9% (95% CI -4.1 to 10.3) for a 10 $\mu g/m^3$ increase in O_3, PM_{10} and NO_2 respectively. Heat effects in the warm season were acute and were followed

by a deficit in mortality three to five days after the exposure. Taking into account this harvesting effect, the estimated 11-day (lag 0–10) increase in dairy cattle mortality for a 1°C increase in mean temperature above the heat threshold (16.8°C) was 2.17% (95% CI 0.11 to 4.28). We also found increases in mortality associated with low temperatures in the warm season. Cold effects were delayed by five days and persisted up to 18 days after the exposure. Over lag 0–25 days, the estimate for a 1°C decrease below the cold threshold (13.9°C) was 4.90% (95% CI 0.40 to 9.60). Results are largely consistent with those from human studies, suggesting that dairy cattle registries might serve as a sensitive sentinel system for the surveillance of environmental health risks.

3. Interrelations between exposures and outcomes

The underlying hypothesis of combining air pollution exposure and urban climate is that these factors interact and significantly affect the health of humans. Interventions and policies may, however, reduce the health impacts, and such benefits can be better estimated than before. Air pollution is believed to change in the future due to changed climate and changes in precursor emissions, and thus a revision of knowledge on the air-borne health effects is needed for the years 2030 and beyond.[57] Improving control over emissions of particulate and gaseous species can improve air quality as well as mitigate climate change.[58]

Our studies suggest that the different environmental risk factors might have independent effects on the health outcomes studied. Although it is unlikely that changes in health outcomes at the moment of the smoking ban implementations are due to sudden changes in exposures other than second hand smoke, analyses were adjusted for air pollution and temperature levels. Likewise, when studying air pollution effects, potential confounding by temperature was taken into account and vice versa when studying temperature effects (in the main analysis or in sensitivity analyses). Adjustments for temperature and/or air pollution were done by means of flexible (non-linear) terms. Similarly, in studies on temperature and air pollution, potential (acute or gradual) changes in health outcomes due to smoke-free legislation (or other factors) were corrected for by including flexible time trend variables into the model (or by matching in case-crossover analyses). However, the effects of highly correlated exposures (such as temperature and

ozone concentrations) are difficult to disentangle and residual confounding by measured or unmeasured exposures may exist.

A distinction should be made between acute (triggering) effects and more gradual effects. Results of chapters 2 and 3 suggest that, in addition to the immediate health effects, smoke-free legislation might also result in more delayed effects. We expect that the former are caused by immediate changes in environmental tobacco smoke exposure, whereas the latter might be due to more gradual changes in active smoking. We did, however, not find an effect of the implementation of the smoke-free legislation on (low) birth weight, nor on the risk of being small-for-gestational-age. Possibly, the risk reduction of preterm birth at the population level attributed to smoking bans is not large enough to translate into an effect on population birth weight. Furthermore, second-hand smoke exposure and extreme temperatures could act as an onset factor or trigger for preterm birth, whereas fetal growth is more importantly affected by its cumulative effect over time. The latter is also suggested by the observation that the association between fetal growth and maternal exposure to PM_{10} appears to be stronger for longer exposure windows than for than shorter windows.

Although it is well accepted that temperature and air pollution act as triggers for human mortality, the number of studies investigating the temporal pattern is more limited, especially for air pollution. Our results support the hypothesis that temperature and air pollution effects are not limited to a forward displacement of mortality (harvesting) among frail individuals. The observation of delayed increases in mortality suggests that air pollution is associated with exacerbation of illness, resulting in an increased recruitment into the pool of individuals at risk of dying.

4. Implications of the presented work for public health

Preterm birth and low birth weight are major causes of neonatal and child morbidity and mortality. Worldwide more than 15 million babies (11%) are born preterm each year,[59] and more than 20 million (15.5%) have a low birth weight.[60] 17% of deaths among children under the age of five are due to preterm birth complications.[61] Next to these short-term effects, preterm birth and birth weight are known to be associated with several health problems throughout life.

According to the Barker hypothesis, suboptimal intrauterine conditions may alter fetal programming during critical periods of growth, causing permanent changes in metabolism and cardiovascular or renal structure and function. Low birth weight may be due to preterm birth, poor fetal growth, or a combination of both, and is associated with increased risk of neurobehavioral problems,[62] and cardiovascular,[63 64] metabolic,[65] and renal disease[64] later in life. Therefore, the public health impact of reductions in the studied birth outcomes is not limited to childhood, but projects into adulthood. The same is true for asthma. Asthma is the most common chronic disease in children[66] and is associated with a reduced lung function and COPD in adults.[67] Also the public health relevance of a reduction in acute myocardial infarction is unambiguous. Acute myocardial infarction and angina are the two leading manifestations of ischemic heart disease, which is the leading cause of death worldwide, causing 7.4 million deaths in 2012.[68]

A recent study suggests that in high-income countries the five most effective preventive measures (maternal smoking cessation, progesterone treatment, cervical cerclage, decreasing non-medically indicated caesarean section and labor induction, and limiting multiple embryo transfer in assisted reproductive technology) could maximally reduce preterm births by 5%.[69] Compared to this estimate, a possible 10% reduction through smoke-free legislation (as estimated in our meta-analysis[16]) is promising.

The public health impact of smoke-free legislation goes beyond the targeted reduction in passive smoking. Restrictions on smoking have made tobacco use less socially acceptable,[70] and have resulted in a decrease in smoking prevalence, a decrease in cigarette consumption among continuing smokers, and a reduction in smoking initation.[71 72] Therefore it is very likely that a reduction in active smoking has contributed to the observed health effects. It is also important to note that despite fears that smoke-free policies would lead to more smoking at home, studies have shown the opposite to be true.[71 73] Strong smoke-free laws have changed the social norms around smoking, leading to reduced smoking at home, thus having a major impact on child health outcomes.

Currently, only 16% of the world population is covered by comprehensive smoke-free laws.[74] Our demonstration of beneficial effects of smoke-free legislation on adult as well as child health provides further support for the continuation and

implementation of smoke-free laws worldwide. Likewise, our finding of detrimental effects of PM_{10} on fetal growth at levels well below current air quality standards (EU limit of 40 µg/m^3 for annual mean) is important for policy-making. As ambient temperatures cannot be modified, our findings indicate that pregnant women should protect themselves from temperature extreme.

On-farm death of adult dairy cows is a significant problem for both economic and animal welfare reasons. Adult cow mortality losses on dairy farms have increased in recent years and there is large uncertainty about exact causes of death in dairy cattle.[75 76] The investigation of dairy cow mortality in relation to environmental risk factors is not only interesting from a veterinary point of view, but might also add to the epidemiological evidence on human health risks. Timely data on dairy cow mortality are readily available from mandatory registries of farm animals and a routine monitoring of these data might serve as an early warning system for the effects of known or new environmental hazards for animal as well as human health.

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Curriculum Vitae

Bianca Cox was born in Sint-Truiden (Belgium) on March 21st 1980. In 1998, she graduated from secondary school at the Amandinacollege in Herk-de-Stad and started her study in Biology at Antwerp University. She followed optional courses in environmental health and did her master thesis in this field. She also followed an academic teaching course at Antwerp University and obtained an additional education in statistics (MSc in Applied Statistics & MSc in Biostatistics) at Hasselt University. After graduation in 2005, Bianca Cox started working as a scientific collaborator at the department of Epidemiology (the current Public Health and Surveillance department) at the Scientific Institute of Public Health (WIV-ISP) in Brussels. There, she was responsible for the monitoring of all-cause mortality in relation to ambient temperature, air pollution and influenza, and she was involved in many other studies. Bianca Cox was also an active member of EU projects on mortality monitoring (EuroMOMO) and health expectancies (EHLEIS and EHEMU). In 2010, she started her PhD in the research unit of Prof. Dr Nawrot at the Centre for Environmental Sciences of Hasselt University. In her PhD she used population-based registries to study the effects of environmental exposures and preventive measures on different health outcomes. One of the studies (on preterm birth and temperature) was embedded within a FP-7 ERA-NET project ACCEPTED (Assessment of Changing Conditions, Environmental Policies, Time-activities, Exposure and Disease).

List of publications

International peer-reviewed publications

1. **Cox B**, Vicedo-Cabrera AM, Gasparrini A, Martens E, Vangronsveld J, Forsberg B, Nawrot TS. Ambient temperature as trigger of preterm delivery in a temperate climate. *Submitted*.

2. Winckelmans E*, **Cox B***, Martens E, Fierens F, Nemery B, Nawrot TS. Fetal growth and maternal exposure to particulate air pollution - More marked effects at lower exposure and modification by gestational duration. *Environmental Research*, *revised version submitted*. *Contributed equally to this work

3. Devos S, **Cox B**, Dhondt S, Nawrot TS, Putman K. Cost saving potential in cardiovascular hospital costs due to reduction in air pollution. *Science of the Total Environment, in press*.

1. Saenen ND, Plusquin M, Bijnens E, Janssen BG, Gyselaers W, **Cox B**, Fierens F, Molenberghs G, Penders J, Vrijens K, De Boever P, Nawrot TS. In Utero Fine Particle Air Pollution and Placental Expression of Genes in the Brain-Derived Neurotrophic Factor Signaling Pathway: An ENVIRONAGE Birth Cohort Study. *Environmental Health Perspectives* 2015.

2. Pieters N, Koppen G, Van Poppel M, De Prins S, **Cox B**, Dons E, Nelen V, Int Panis L, Plusquin M, Schoeters G, Nawrot TS. Blood Pressure and Same-Day Exposure to Air Pollution at School: Associations with Nano-Sized to Coarse PM in Children. *Environmental Health Perspectives* 2015.

3. Pieters N, Janssen BG, Valeri L, **Cox B**, Cuypers A, Dewitte H, Plusquin M, Smeets K, Nawrot TS. Molecular responses in the telomere-mitochondrial axis of ageing in the elderly: A candidate gene approach. *Mechanisms of Ageing and Development* 2015;145:51-7.

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Books

1. Debacker N, Temme L, **Cox B**. Belgian food consumption survey 2004. Eating patterns among the Belgian population aged 15 years and over. Brussels, Belgium: WIV-ISP, 2008.

Reports

1. Koppen G, De Prins S, **Cox B**, Dons E, Van de Mieroop E, Nelen V, Nawrot T, Van Poppel M. Kwantitatieve inschatting van blootstelling aan en gezondheidseffecten van verkeersemissies in Vlaanderen, met speciale aandacht voor Ultrafijne Partikels (UFP). Pilootproject in geselecteerde hotspot(s): Health Effects of Air Pollution in Antwerp Schools (HEAPS). Mol, Belgium: VITO in opdracht van het Departement Leefmilieu, Natuur en Energie (LNE) & het Agentschap voor Zorg en Gezondheid (AZG), 2012.
2. Den Hond E, Baeyens W, **Cox B**, De Sutter P, D'Hooghe T, Kaufman JM, Nawrot T, Ombelet W, Tournaye H, Vandendael A, Van Larebeke N. Associatie tussen milieublootstelling en fertiliteit: een case-control studie bij subfertiele mannen en een interim analyse bij subfertiele vrouwen. Mol, Belgium: VITO in opdracht van het Steunpunt Milieu en Gezondheid, 2012.
3. **Cox B**, Wuillaume F, Maes S. Algemene mortaliteit tijdens het griepseizoen 2007-2008. In: Surveillance van Griep in België. Seizoen 2007-2008. Jaarrapport. Brussels, Belgium: WIV-ISP, Nationaal Influenza Centrum, 2008 (REF: D/2008/2505/57).
4. **Cox B**, Wuillaume F, Maes S, Van Oyen H. Mortality by region during the hot summers of 2003 and 2006, Belgian Mortality Monitoring (Be-MOMO). Brussels, Belgium: WIV-ISP, 2008 (REF: D/2008/2505/41).
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6. Maes S, **Cox B**, Wuillaume F. Mortaliteit binnen de globale populatie tijdens de registratieperiode van ILI en ALI. In: Surveillance van Griep in België. Seizoen 2006-2007. Jaarrapport. Brussels, Belgium: WIV-ISP, Nationaal Influenza Centrum, 2008 (REF: D/2008/2505/05).
7. Van der Heyden J, Kunst A, Esnaola S, Borrell C, Kalediene R, **Cox B**, Schaap M, Mackenbach J, Leinsalu M, Van Oyen H. Socio-economic inequalities in lung cancer mortality in Europe: an update. In: Tackling Health Inequalities in Europe: an Integrated Approach. Eurothine Final Report. Rotterdam, the Netherlands: Erasmus University, 2007.
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Abstracts

1. **Cox B**, Vicedo-Cabrera AM, Gasparrini A, Martens E, Vangronsveld J, Forsberg B, Nawrot TS. Ambient temperature as trigger of preterm delivery in a temperate climate. Young Researchers Conference on Environmental Epidemiology (ISEE-EUROPE 2014), Barcelona, Spain, 20-21 Oct 2014 (oral presentation).

2. Winckelmans E, **Cox B**, Martens E, Nemery B, Nawrot TS. The shape of the association between fetal growth and maternal particulate air pollution and effect modification by gestational duration. Young Researchers Conference on Environmental Epidemiology (ISEE-EUROPE 2014), Barcelona, Spain, 20-21 Oct 2014 (oral presentation).

3. Saenen ND, Plusquin M, Bijnens E, Janssen BG, Gyselaers W, **Cox B**, Fierens F, Molenberghs G, Penders J, Vrijens K, De Boever P, Nawrot TS. In utero Exposure to Fine Particle Air Pollution: Using Placental Gene Expression of the BDNF Signaling Pathway to Explore Fetal Neural Development. Young Researchers Conference on Environmental Epidemiology (ISEE-EUROPE 2014), Barcelona, Spain, 20-21 Oct 2014 (oral presentation).

4. Louwies T, De Boever P, **Cox B**, Dons E, Penders J, Provost E, Int Panis L, Nawrot TS. Blood pressure changes in association with personal black carbon exposure are not mediated through microcirculatory responses. Young Researchers Conference on Environmental Epidemiology (ISEE-EUROPE 2014), Barcelona, Spain, 20-21 Oct 2014 (poster presentation).

5. Pieters N, Janssen BG, Smeets K, **Cox B**, Cuypers A, Dewitte H, Fierens F, Plusquin M, Nawrot TS. Long-term particulate air pollution in association with the mitochondrial-telomere axis of ageing. Young Researchers Conference on Environmental Epidemiology (ISEE-EUROPE 2014), Barcelona, Spain, 20-21 Oct 2014 (poster presentation).

6. Deboever P, Louwies T, Kounalakis SN, **Cox B**, Jaki Mekjavic P, Nawrot TS, Eiken O, Mekjavic IB. PlanHab: In vivo retinal images for a non-invasive analysis of the microcirculation during hypoxia and unloading/inactivity. 35th Annual International Gravitational Physiology Meeting, Waterloo, Canada, 16-20 Jun 2014 (oral presentation).

7. Den Hond E, Baeyens W, **Cox B**, De Sutter P, Nawrot T, Ombelet W, Tournaye H, Van Larebeke N, D'Hooghe T. Human exposure to chlorinated and brominated compounds is associated with increased risk for subfertility. 49th Congress of the European Societies of Toxicology (EUROTOX), Interlaken, Switzerland, 1-4 Sep 2013 (poster presentation).

8. **Cox B**, Martens E, Nemery B, Vangronsveld J, Nawrot TS. Impact of a stepwise introduction of smoke-free legislation on the rate of preterm births: analysis of routinely collected birth data. International Society for Environmental Epidemiology (ISEE) annual conference, Basel, Switzerland, 19-23 Aug 2013 (oral presentation by Nawrot TS).

9. Provost E, Chaumont A, Kicinski M, **Cox B**, Fierens F, Bernard A, Nawrot TS. Serum levels of Clara cell protein and short- and long-term exposure to particulate air pollution in adolescents. International Society for Environmental Epidemiology (ISEE) annual conference, Basel, Switzerland, 19-23 Aug 2013 (oral presentation).

10. Bijnens E, Pieters N, Dewitte H, **Cox B**, Janssen B, Saenen N, Dons E, Zeegers MP, Int Panis L, Nawrot TS. Host and environmental predictors of exhaled breath temperature in the elderly. International Society for Environmental Epidemiology (ISEE) annual conference, Basel, Switzerland, 19-23 Aug 2013 (oral presentation).

11. Pieters N, Koppen G, Plusquin M, De Prins S, **Cox B**, Nelen V, Int Panis L, Schoeters G, Van Poppel M, Nawrot TS. Children's blood pressure in association with exposure to ultrafine particulate air pollution at school. International Society for Environmental Epidemiology (ISEE) annual conference, Basel, Switzerland, 19-23 Aug 2013 (oral presentation).

12. De Prins P, Van Poppel M, Dons E, Int Panis L, Van de Mieroop E, **Cox B**, Nawrot TS, Schoeters G, Koppen G. Heaps study results: health effects of air pollution in Antwerp schools. International Society for Environmental Epidemiology (ISEE) annual conference, Basel, Switzerland, 19-23 Aug 2013 (poster presentation).

13. De Prins S, Koppen G, Jacobs G, Dons E, Van de Mieroop E, Nelen V, Fierens F, De Boever P, **Cox B**, Nawrot TS, Schoeters G. Influence of air pollution on global DNA methylation in healthy adults: a seasonal follow-up. International Society for Environmental Epidemiology (ISEE) annual conference, Basel, Switzerland, 19-23 Aug 2013 (poster presentation).

14. **Cox B**, Nawrot TS. The impact of a stepwise introduction of smoke-free legislation on the rate of preterm births. Developmental Origins of Health and Disease (DOHaD) 2012 satellite meeting: New developments in developmental epidemiology, Rotterdam, the Netherlands, 6-7 Dec 2012 (oral presentation by Nawrot TS).

15. De Prins S, Jacobs G, Fierens F, Dons E, Nelen V, Van de Mieroop E, **Cox B**, Constandt K, Nawrot T, Schoeters G, Koppen G. Influence of air pollution on oxidative stress and inflammation in healthy adults: a seasonal follow-up. BIOMAQ (Biomonitoring of air quality with plants, animals and humans) conference, Antwerp, Belgium, 12-14 Nov 2012 (oral presentation).

16. **Cox B**, Martens E, Nemery B, Vangronsveld J, Nawrot TS. Ambient temperature and its daily fluctuation as trigger of preterm delivery in a temperate climate. International Society for Environmental Epidemiology (ISEE) annual conference, Columbia, South-Carolina, 26-30 Aug 2012 (poster presentation).

17. Pieters N, Koppen G, De Prins S, Spruyt M, Van De Weghe H, Nelen V, **Cox B**, Smeets K, Cuypers A, Schoeters G, Nawrot TS. Decreased mitochondrial DNA content in association with indoor exposure to polyaromatic hydrocarbons. International Society for Environmental Epidemiology (ISEE) annual conference, Columbia, South-Carolina, 26-30 Aug 2012 (oral presentation).

18. Pieters N, Plusquin M, **Cox B**, Kicinski M, Vangronsveld J, Nawrot TS. An epidemiological appraisal of the association between heart rate variability and particulate air pollution: a meta-analysis. International Society for Environmental Epidemiology (ISEE) annual conference, Columbia, South-Carolina, 26-30 Aug 2012 (oral presentation).

19. Janssen B, Munters E, Pieters N, Smeets K, **Cox B**, Cuypers A, Fierens F, Penders J, Vangronsveld J, Gyselaers W, Nawrot TS. Decreased placental mitochondrial DNA-content in response to particulate air pollution during in utero life. International Society for Environmental Epidemiology (ISEE) annual conference, Columbia, South-Carolina, 26-30 Aug 2012 (poster presentation).

20. De Prins S, Koppen G, **Cox B**, Nelen V, Fierens F, Constandt K, Nawrot T, Schoeters G. Influence of traffic-related air pollution on biomarkers of oxidative stress and inflammation in healthy adults: seasonal follow-up. International Society for Environmental Epidemiology (ISEE) annual conference, Barcelona, Spain, 13-16 Sep 2011 (poster presentation).

21. **Cox B**, Wuillaume F. Pandemic mortality and pilot experiences in Belgium. EuroMOMO plenary meeting, Berlin, Germany, 27 Apr 2010 (oral presentation).

22. **Cox B**, Wuillaume F. Mortality monitoring method used in Belgium. EuroMOMO annual meeting, Rome, Italy, 2-6 Mar 2009 (oral presentation).

23. Drieskens S, Tafforeau J, Van Oyen H, **Cox B**, Lafontaine MF. Interactive web-based applications to optimize the accessibility to Belgian health statistics. 16th European Public Health Association (EUPHA) Conference, Lisbon, Portugal, 5-8 Nov 2008 (oral presentation).

24. Van Oyen H, Van der Heyden J, **Cox B**, Perenboom R, Jagger C. Monitoring population disability: evaluation of a new Global Activity Limitation Indicator (GALI). Conference Europe for patients, Paris, France, 13-14 Oct 2008 (poster presentation).

25. **Cox B**, Van Oyen H, Vandevijvere S. Methods for estimation of usual intake. AgroStat 2008: 10th European Symposium on Statistical Methods for the Food Industry, Louvain-la-Neuve, Belgium, 23-25 Jan 2008 (poster presentation).

26. Van der Heyden J, Kunst A, **Cox B**, Deboosere P, Van Oyen H. Socioeconomic inequalities in lung cancer mortality in Europe. Results from the EUROTHINE project. 9th Symposium of Public Health and the End of Life, Brussels, Belgium, 14 Dec 2007 (oral presentation).

27. **Cox B**, Maes S. Mortality surveillance in Belgium: Results summer 2006. European Conference on heat-waves, Bonn, Germany, 22-23 Mar 2007 (poster presentation).

28. Van Oyen H, **Cox B**. Trends in disability-free life expectancy in Belgium between 1997 and 2004. 18th REVES meeting: Differential trends in health expectancy, implications for the future, Amsterdam, the Netherlands, 29-31 May 2006 (oral presentation).

Awards

Dr Luc Broeckaert prize 2015 of the Royal Academy of Medicine of Belgium, with a work, entitled 'Health effects of smoke-free legislation'
(Dr. Luc Broeckaert en familie 2015 voor geneeskundig wetenschappelijk onderzoek, gericht op preventie en praktijk, Koninklijke Academie voor Geneeskunde van België, met een werk, getiteld: 'Gezondheidseffecten van rookvrije wetgevingen')