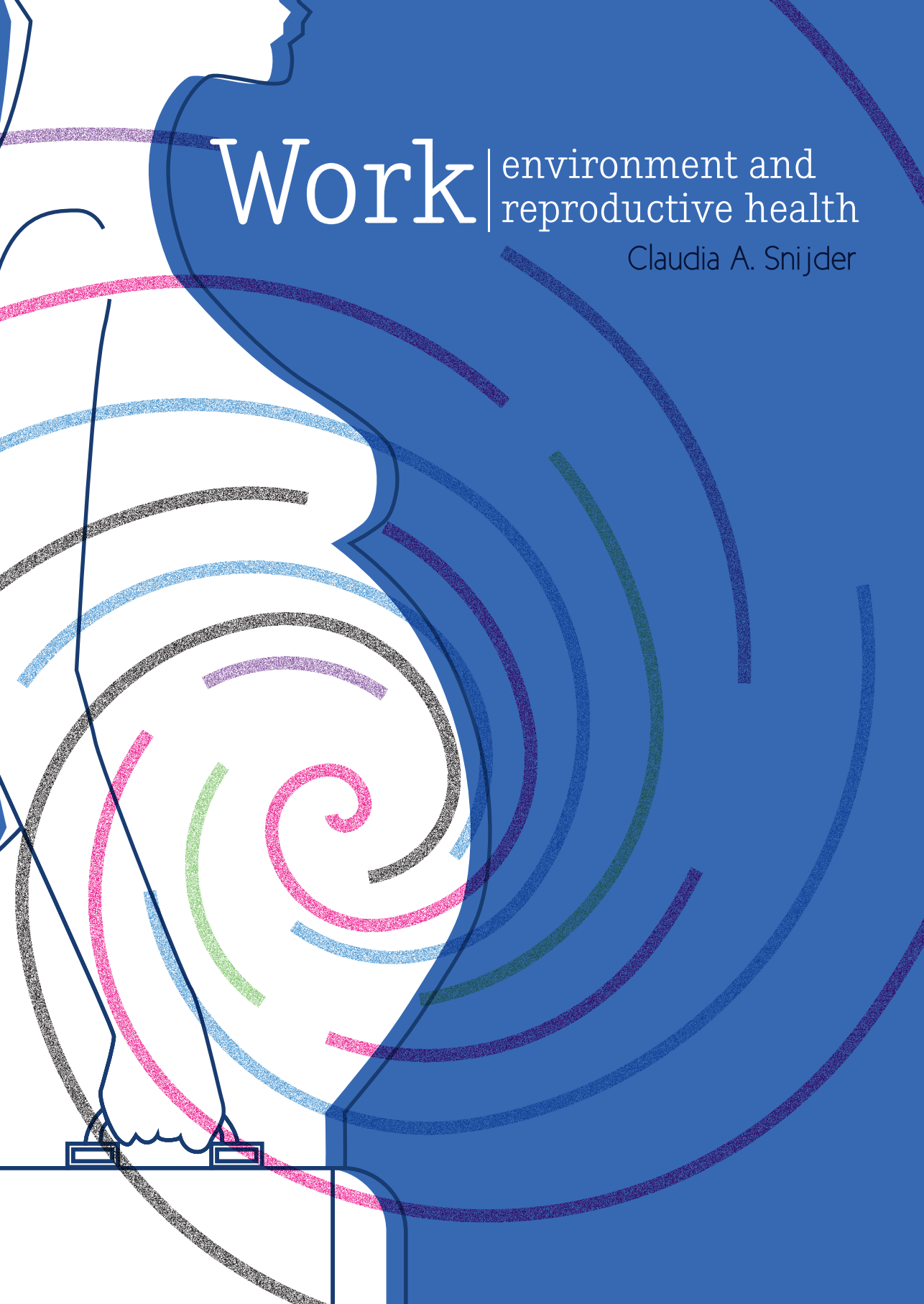
Work | environment and reproductive health

Claudia A. Snijder



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PART 1

WORK AND REPRODUCTIVE HEALTH

1. GENERAL INTRODUCTION

With the increasing labour force participation among women in Western countries, many women will work during their reproductive years. This will increase the likelihood that women during their reproductive years will be exposed to a variety of risk factors at work that may effect their reproductive abilities and the outcome of their pregnancy, such as spontaneous abortion, hypertensive disorders, intrauterine growth restriction, and adverse birth outcomes.[1,2] Occupational exposures may also interact with foetal development, resulting in health effects in the offspring, such as congenital malformations and neurobehavioural disorders at young age.[3-6] For several work-related risk factors the associations with reproductive effects are well established and translated into legislation, such as mandatory provisions for pregnant women preparing antineoplastic drugs or being exposed to lead.[7] However, for many other work-related risk factors, the scientific evidence is less consistent. Work-related risk factors can be divided into chemical agents such as metals, solvents, pesticides, physical agents such as radiation and noise, and ergonomic factors such as heavy workload, shift work, and psychosocial stress.[8]

Research into occupational exposures and effects on the reproductive system has made important scientific contributions in the past years. Early studies focussed on the possible effects on pregnancy and the foetus rather than on the reproductive health of women. Later, it was realised that reproductive toxins may also induce hormonal alterations affecting other aspects of reproductive health such as menstrual cycle disorders, and fertility. Attention has shifted to the entire spectrum of occupational hazards among women and the reproductive health of both genders. Since the 1950s, adverse trends in the reproductive health of certain wildlife populations have been observed. Concerns over the release of an array of hormone mimicking chemicals into the environment were raised when populations of certain wildlife species started to decline as result of individuals within the population exhibiting strange behaviour or displaying physical malformations.[9] With the recognition that even low concentrations of endocrine disrupting substances can devastate the health and fertility of wildlife populations, their effects on human reproductive health have become a major concern.

2. ENDOCRINE DISRUPTORS

An endocrine disruptor (ED) is an exogenous substance or mixture that alter(s) the normal functioning of the endocrine system and causes adverse health effects in an intact organism, or its progeny.[10,11] Rachael Carson was among the first to report the endocrine disrupting abilities of man made chemicals, when she observed a decline in birds of prey populations. Egg shell thinning and other reproductive disorders were ascribed to dichlorodiphenyltrichloroethane (DDT) exposure, and it was noted that the species most affected were at the top of

the food chain, due to the bioaccumulative properties of organochlorine chemicals.[12] EDs are widely spread in the environment and display estrogenic, anti-estrogenic or anti-androgenic activity. The main targets of ED chemicals are the homeostasis of sex steroids and the thyroid. EDs are a broad and diverse group of chemicals, as regards use, chemical structure and modes of action. They include a long list, such as persistent bioaccumulative pollutants (dioxins, DDT), chemicals used in plant or animal food production (several types of pesticides), and compounds widely used in industry or consumer products (phthalates, Bisphenol A).[13] Potential routes for exposure are food products, the general environment, consumer products, and occupation.

3. CHEMICALS, ENDOCRINE DISRUPTORS AND REPRODUCTIVE HEALTH

3.1 Reproductive toxicity

Reproductive toxicity is defined as a condition causing deleterious response in the post-pubescent male or female manifested by the interference with normal physiological processes or regulatory mechanisms, organ functioning, or the genetic integrity of the sperm or egg cells. In the human population, the alleged adverse reproductive health effects of chemicals or exogenous hormone-like substances include reduced number and deterioration in the quality of sperm, reduced fertility, delayed development and abnormality of the reproductive organs, increased incidence of testicular and breast cancers, and possible cardiovascular effects.

Fecundity, the capacity of couples to conceive and have children, depends on numerous biological processes including spermatogenesis, oogenesis, transport of gametes, fertilisation of the oocyte, implantation of the embryo, and the development of the foetus thereafter.[14] Hence, the time it takes to become pregnant since actively trying to conceive, time to pregnancy (TTP), was reported by Baird et al. as a good measure for estimating fecundity[15,16] and has recently been described as a sensitive and feasible measure for studying occupational exposures as well as for monitoring of fecundity.[17] TTP as outcome has been extensively used in epidemiological studies to detect the effects of occupational exposures.[16,18-20] To gain more insight in how EDs influence human fecundity, studies assessing exposure to EDs and TTP are valuable. Currently, the number of studies relating occupational ED exposure to TTP is limited. Several studies investigated distinct exposures in specific occupations in relation to TTP, most notably pesticide exposure among greenhouse and agricultural workers. Reviews by Roeleveld et al., Hanke et al., and Bretveld et al. found limited evidence for an influence of exposure to pesticides among fathers or mothers on reproduction.[21-23] However, the focus on either male or female exposure and subsequent lack of adjustment for a partner's exposure make the results difficult to interpret.[1]

Effects of chemicals, possibly through endocrine disrupting mechanisms, on sperm quality have also been extensively studied. A report by Carlsen et al. suggested a possible global decrease in sperm concentration.[24] The authors conducted a meta-analysis of 61 studies involving 14947 men from 23 different countries. They found that sperm concentration had dropped from $113x10^6$ spermatozoa per millilitre of ejaculate in 1940 to $66x10^6$ spermatozoa per millilitre in 1990. Since Carlsen et al. reported that mean sperm counts decreased by 50% during the second half of the last century and suggested that this decline in sperm quality and the increasing prevalence of genitourinary abnormalities may have a common environmental aetiology, there has been widespread anxiety about the effects of environmental pollutants on human reproduction. These concerns are contradicted by the results of various population studies in Europe and the US on secular trends in fecundity, indicating that population fecundity has either improved or remained unchanged over the past 30–40 years. Sperm concentration in Toulouse (France) has remained stable[25] but it has dropped over time in Paris.[26] In Finland, sperm concentrations increased[27] while remaining stable in Belgium[28] and the United States[29] and decreasing in the United Kingdom.[30] So, the results of sperm studies are diverse, complex, and difficult to interpret. The claims that population fecundity is declining and that environmental pollutants are involved, can neither be confirmed nor rejected.[17]

EDs have also been linked to cryptorchidism and hypospadia and its increased incidence in recent years. EDs supposed to play a role in cryptorchidism mimic the action of hormones involved in the testicular decent, and act mainly as estrogens and anti-androgens. According to the testicular dysgenesis syndrome (TDS) theory presented by Skakkebaek et al., cryptorchidism, hypospadias, testicular cancer, and spermatogenic impairment share the same risk factors and have a foetal origin, caused by a combination of genetic and environmental factors, including EDs.[31] This hypothesis is supported by evidence that exogenous estrogens and anti-androgens cause disorders of genital development in animals.[32] One of the main facts that prompted the formulation of this theory was the evidence that boys born to women who had been exposed to diethylstilbestrol (DES) in early pregnancy had an increased incidence of cryptorchidism and other genital defects.[33] Recently, some evidence was presented that over-the-counter mild analgesics may also increase the risk of cryptorchidism in the offspring.[34] This study showed that paracetamol, even at low plasma concentrations of 1μM, is a potent inhibitor of testosterone production, reducing anogenital distance and testosterone production in rats. Experimental rat models have shown that normal androgen action during a critical male programming window is crucial for the programming of the testis decent.[35] These experimental observations have found echoes in human observational studies,[34,36] but further research is urgently needed to corroborate or refute these recent findings. Since the proportion of women using mild analgesics during pregnancy is high, population impact may be substantially.

3.2 Developmental toxicity

Developmental toxicity is a condition producing adverse effects on the developing organism reflected in prenatal or early postnatal death, altered growth, structural abnormalities and functional deficits.[37]

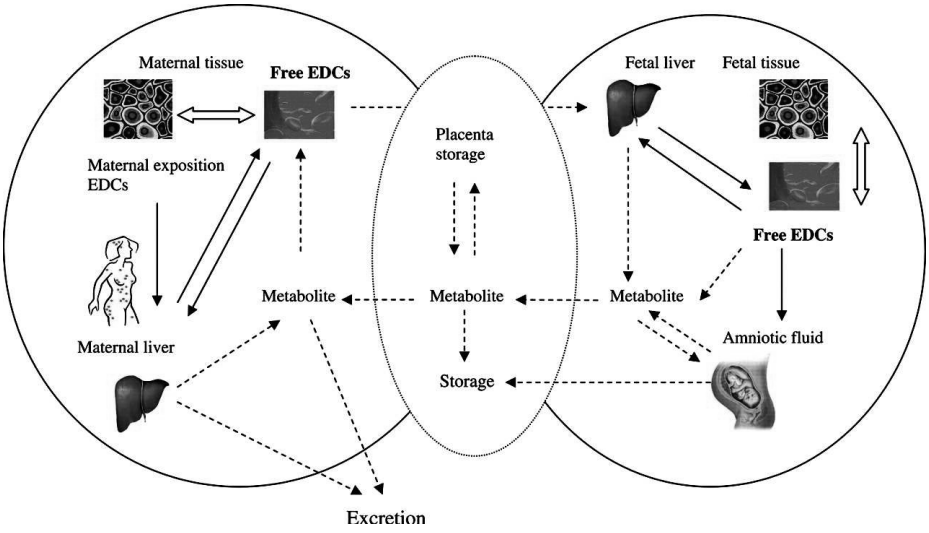
The Health Council of the Netherlands has listed the possible effects of *in utero* exposure to chemicals with ED properties, which are summarised below:[38,39]

- Abnormal development of the reproductive system (cryptorchidism, hypospadia).
- Cancer promotion (testicular, cervical, and uterine cancers).
- Decreased sperm concentration or quality, and reduced spermatogenesis.
- Abnormal development of the Central Nervous System (neurological, cognitive and behavioural disorders).
- Other developmental abnormalities (shortened pregnancy, low birth weight, disturbed hormonal regulation, sex ratio effects).

The aforementioned effects of chemicals or EDs on reproductive health have increased concerns about effects of occupational exposures on pregnancy outcome and foetal development.[11,40-42] These concerns come from an increased understanding that the foetus is extremely sensitive during certain critical windows of development. Windows of sensitivity exist for many systems - respiratory, immune, reproductive, nervous, cardiovascular, and endocrine - as well as for general growth and later outcomes such as childhood and adult onset cancers.[43] The placenta was at one time thought to offer a highly effective barrier minimising contaminant exposure, although research in recent decades has documented that it is far from impenetrable.[44,45] Chemicals can cross the placenta and enter the foetus, and a number of chemicals measured in maternal urine and serum have also been found in amniotic fluid, cord blood, and meconium.[46] This is also illustrated in Figure 1, whereby transplacental transfer is well recognised for most EDs. In some cases, the placenta may actually magnify maternal exposures, depending on mechanism of transport across the placenta, protein binding of the chemical in maternal and foetal serum and physicochemical characteristics of the agent. Cord blood levels of methyl mercury, for example, have been shown to be nearly two times higher than corresponding maternal levels.[47] A recent study by Woodruff et al. showed that pregnant women in the US were exposed to multiple chemicals.[48] The mechanism by which chemicals affect foetal development are not completely understood. Direct toxic effects may occur when normal processes such as differentiation, mitosis, meiosis, intracellular communication, DNA repair are altered, but also indirect toxic effects may occur, the underlying routes are not yet clarified.

Adverse birth outcomes, such as low birth weight, small-for-gestational-age, and preterm delivery, are major determinants of infant mortality and morbidity.[49,50] Environmental exposures and lifestyle behaviours, acting at different stages of foetal development, are held partly responsible for adverse birth outcomes.[5,51-54] Parental occupation[55,56] and occupational

FIGURE 1. Schematic presentation of EDs distribution in the materno-foeto-placental unit



From: Caserta et al. 2011[13]

Transplacental transfer is well recognised for most EDs: a synthesis of the exposure pathway of the placental-foetal unit is shown in Figure 1.

exposures to chemicals such as pesticides,[57,58] phthalates,[59] and metals,[60,61] have also been associated with adverse birth outcomes. The effects of occupational risk factors, including exposure to chemicals or EDs, on birth outcomes have been studied extensively, but studies on the effects of these risk factors on intrauterine growth are scarce. Since occupational exposure to chemicals with ED properties may affect foetal organ development as shown by its associations with hypospadias and cryptorchidism,[31] it is hypothesised that chemicals may also delay foetal growth from early pregnancy onwards. Information on the effects of maternal occupational exposure to chemicals during pregnancy on important parameters of foetal growth during pregnancy, such as estimated foetal weight, foetal head circumference, and foetal length, may provide insight in how to counsel pregnant women occupationally exposed to certain chemicals.

4. PHYSICALLY DEMANDING WORK

As previously mentioned, women constitute a substantial part of the labour force in the European Union (EU). In 2010, about 58% of the women aged between 15-64 years had paid employment, which was a substantial increase from 54% in 2002.[62] With the increasing labour force participation among women in European countries, the likelihood that women will be exposed to a variety of chemical, physical, and psychological risk factors at work during

pregnancy will also increase.[63] Although women in paid employment have better pregnancy outcomes than those without paid jobs,[64,65] certain work-related risk factors, such as exposure to chemicals,[51] and physically demanding work[66] may adversely influence pregnancy outcome. In theory, long working hours, prolonged standing, heavy lifting or unusual workload may pose a number of treats to the pregnant worker. For example, the high demand of uterine and placental blood flow in the third trimester could limit reserve capacity for vigorous exercise, the gravid uterus could limit venous return and cardiac output, especially in those who stand, and the release of catecholamines could increase the risk of premature contractions.[67] Furthermore, heavy physical work is thought to reduce the blood flow to the uterus and placenta, thereby reducing the availability of oxygen and nutrients for the foetus.[68,69] Practical management of pregnant women is made more difficult, because the activities of concern, although suspected of being hazardous, could also be beneficial.

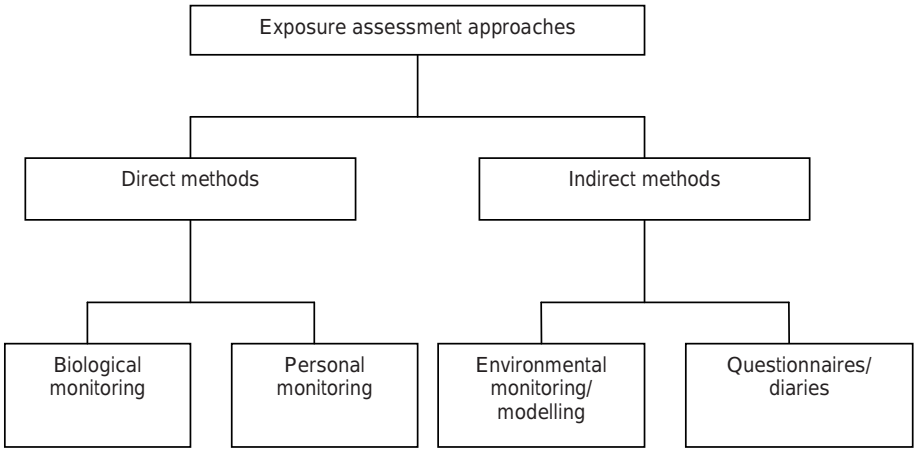
To help clarify the way forward, epidemiological studies are needed relating physically demanding work not only to adverse birth outcomes, but also to effects on intrauterine growth, and other pregnancy complications, such as hypertensive disorders during pregnancy.

5. EXPOSURE ASSESSMENT

Exposure assessment, the study of the distribution and determinants of substances or factors affecting human health, is an important issue in occupational epidemiology. Exposure can be classified, measured, or modelled and different tools are available for this, such as questionnaires, air pollution monitors, and statistical techniques, respectively. The methods are often classified as direct and indirect (Figure 2). Time and location play an important role. Traditionally, exposure in the workplace tends to be higher than in the general environment and the duration of exposure is generally shorter. Quantification of the association between exposure and adverse human health effects requires the use of exposure estimates, which are accurate, precise and biologically relevant for the critical exposure period, and show a range of exposure levels in the population under study. Subjects in an epidemiological study can be classified for their exposure with different strategies. This can be, for example, achieved by: 1) expert assessment, a member of the research team decides based on prior knowledge, whether the subject in the study is exposed on unexposed, and 2) self-assessment by questionnaire, the subject fills out a questionnaire with questions whether he/she is exposed to particular substances, and 3) measurements, which are a more objective way to assess exposure, important for obtaining information on concentration, for example uptake levels of the substance in the body estimated by biomonitoring.

All these different approaches are not exclusive and often combined to obtain the best exposure index. In occupational epidemiology 'job title' is also frequently used as exposure surrogate. However, it is important to remember that the use of surrogates may lead to

FIGURE 2. Different approaches to human exposure assessment[70]



attenuation in risk estimates. A related issue is that mothers or fathers are often exposed to a number of chemicals simultaneously. In epidemiological studies, it is often not possible and feasible to obtain detailed exposure information on each subject in the study. For example, in a large cohort study, it is not feasible to take measurements from each subject for a variety of chemicals. In this case, it is desirable to carry out a small validation study in a subset. The Job-Exposure-Matrix (JEM), an instrument for exposure assessment, list occupations and/or industries on one axis, and exposure agents on the other, and the cells of the matrix present the probability of exposure to a specific agent in a specific job. In large cohort studies, the JEM is a valuable tool for exposure assessment, since job title and job description are usually recalled quite easily. To estimate the exposure to EDs, a new updated JEM has been published recently, and this JEM is specific for the Dutch work environment. With this updated JEM it is possible to estimate the 'probable' and 'possible' exposure to various chemicals with ED properties. However, the characterisation of exposure in the JEM must be interpreted as exposure probabilities, which are only a crude measure of exposure, which have to be interpreted with caution. Furthermore, the JEM does not contain specific chemicals, but only contains broad groups of chemicals, and the mechanisms of action can vary between specific chemicals in a group. A major drawback of JEMs is that they do not account for variability in tasks and working environments within job titles. However, from the task description, it may become clear that some subjects within a specific job title, for example subjects who have odd jobs around a farm (feeding animals) are less likely to be exposed to pesticides. In this context it is very important to validate the JEM for certain groups of chemicals, for example with biomonitoring. Urine of women characterised as exposed by the JEM can be analysed for metabolites, and compared to non-exposed women.

5.1 Biomonitoring

Biological monitoring is the analysis of human biological samples, which may include, for example, exhaled breath, urine, or blood for a particular substance of interest and/or its metabolites to provide an index of exposure and/or dose. Advantages of biological monitoring compared to personal exposure monitoring are: 1) biological monitoring enables estimation of uptake through all exposure routes, 2) exposure may fluctuate widely over time, biomonitoring could provide information on long term exposure when the biological half-life is sufficiently long, and 3) individual differences are known to exist between subjects, these differences could be reflected in biomonitoring results. But feasibility issues, costs, and time may restrict biological monitoring in epidemiological studies and it is rare that biological samples can be obtained from the entire study population, particularly in large epidemiological studies.

The main goals of many ongoing biomonitoring studies in the general population include identifying exposures of potential concern and setting priorities among chemicals for further research and evaluation. Health based values for assessment of human biomonitoring data need to be developed, so that biomonitoring data can be evaluated across chemicals and populations.[71] Efforts to date have resulted in the publication of human biomonitoring values for more than 80 chemicals. Nowadays, the values available for the general population, and for pregnant women can be used in epidemiological studies to assess the impact of chemicals on various health outcomes.

6. CORE STUDY MATERIAL IN THIS THESIS

6.1 Methods and data source

Almost all of the studies were embedded within the Generation R Study, except for one study, the HAVEN Study. The Generation R Study is a prospective population-based cohort study conducted in Rotterdam, The Netherlands, which was designed to identify early environmental and genetic causes of normal and abnormal growth, development and health from foetal life until young adulthood.[72] Pregnant women with a delivery date between April 2002 and January 2006 were invited to participate. While enrolment ideally took place in early pregnancy, it was also possible after birth of the child. Detailed measurements were planned in early pregnancy (<18 weeks of gestation), mid-pregnancy (18-25 weeks of gestation), and late pregnancy (>25 weeks of gestation) and were performed using ultrasound, physical examinations, biological samples and questionnaires. In total, 9778 women were included, of whom 8880 during pregnancy and another 898 at birth of their child.

For the majority of studies in this thesis we used information collected by questionnaire in mid-pregnancy, which contained questions on the current economic status, work status, date of starting/stopping work, working hours per week, job title, a description of the work tasks, and name of employer. Furthermore, nine questions from the Dutch Musculoskeletal

Questionnaire which concerned long periods of standing, long periods of sitting, long periods of working behind a computer screen, long periods of walking, long periods of working in a warm environment, lifting of heavy loads (>5 and >25 kilograms), long periods of driving and night shifts.[73] These question were followed by a question on self-reported exposure to several types of chemicals.[64]

This study provides ample information to study research questions regarding the influence of occupation on pregnancy. Furthermore, maternal urine was available for biomonitoring. Biological materials, including urine, have been collected in early, mid and late pregnancy and at birth. Urine samples (65ml) were added to the data collection between February 2004 and November 2005.[74] Among 2000 women, urine samples have been collected three times during pregnancy and an additional 1000 women have at least one urine sample.

The HAVEN study is a case-control family study, designed to investigate determinants in the pathogenesis and prevention of congenital heart defects (CHDs). Recruitment of case and control children took place between June 2003 and January 2010 and case children with CHD were enrolled with both parents from four university medical centres, the Erasmus Medical Centre in Rotterdam, Leiden University Medical Centre in Leiden, VU University Medical Centre in Amsterdam, and Amsterdam Medical Centre, the Netherlands. Children with CHD diagnosed in the first 16 months after birth by paediatric cardiologists, were identified from the hospital registry, and invited to participate. Diagnoses were confirmed by echocardiography and/or cardiac catheterisation and/or surgery. Healthy control children, without any major congenital malformation, were ascertained in regular health checks by child physicians, and both parents were randomly selected from medical records from child health centres and invited to participate. At the fixed study moment, 17 months after delivery, case and control families visited the hospital for the standardised collection of information on general characteristics and outcomes.[75-77] The information for the present study was collected in the questionnaire, filled out approximately 17 months after child birth. This questionnaire contained questions on the current economic status, job title, and contained a description of the work tasks.

6.2 CONTAMED project

CONTAMED is an EU-funded project, from the European Union's Seventh Framework Programme (FP7) for Research and Technology Development. This project is coordinated by Professor Andreas Kortenkamp from the School of Pharmacy, University of London. It brings together leading European research teams in toxicology, reproductive biology, endocrinology, epidemiology, metabolomics, chemical analysis and chemicals regulation.

CONTAMED stands for contaminant mixtures and human reproductive health – novel strategies for health impact and risk assessment of EDs. This project aims to make a link between

epidemiological observations and laboratory studies. We will explore the hypothesis that combined exposure to EDs in foetal life may lead to adverse delayed impacts on human reproductive health.

Generation R participates in work package 6, the epidemiological analysis of case-control studies using biomarkers for cumulative ED exposure. The aim is to explore associations between cumulative ED exposure and the risk of congenital urogenital malformations. Using a case-control study design, the objective is to assess whether mothers of sons with cryptorchidism or hypospadia had higher urinary concentrations of ED during pregnancy in their urine. Furthermore, since multiple maternal urine samples are available across different trimesters of pregnancy, this will yield important information about the variability of exposure over time and during the most critical time window of exposure.

The other studies presented in this thesis are closely related to this hypothesis; ED exposure may impose different effects on human reproduction, including effects on fertility, and the development of the foetus during pregnancy.

7. AIMS

The overall aim of this thesis is to study the influence of work-related and environmental risk factors on several aspects of human reproduction, such as fertility, foetal growth and development, pregnancy complications, and congenital malformations.

The specific aims are:

1. To study the influence of occupational exposure to chemicals on reproduction, specifically fecundity, intrauterine growth, hypertensive disorders during pregnancy, and birth outcomes.
2. To study the influence of physically demanding work on intrauterine growth, hypertensive disorders during pregnancy, and birth outcomes.
3. To study the relation between exposure to EDs and the occurrence of congenital malformations, including congenital heart defects and male reproductive tract abnormalities, such as cryptorchidism and hypospadia.

8. OUTLINE

In **Part 2**, studies addressing the first aim, namely the relationship between occupational exposure to chemicals and the effects on human reproduction are presented. **Chapter 2.1** summarises the literature regarding occupational exposure to chemicals and time to pregnancy.

The influence of (occupational) exposure to chemicals on time to pregnancy, intrauterine growth, and congenital malformations are presented in **Chapters 2.2**, **2.3**, **2.4**, and **2.5**. **Part 3** addresses the second study aim, and focusses on the association between physically demanding work, intrauterine growth, pregnancy complications such as hypertensive disorders, and adverse birth outcomes. **Part 4** covers the last study aim. **Chapter 4.1** studies the association between maternal exposure to mild analgesics during pregnancy, in particularly paracetamol, which acts as ED by inhibiting the testosterone production, on the occurrence of cryptorchidism and hypospadia in their offspring. **Part 5** provides an overall discussion of the main findings in this thesis, including recommendations for further research, and implications for policy and practice.

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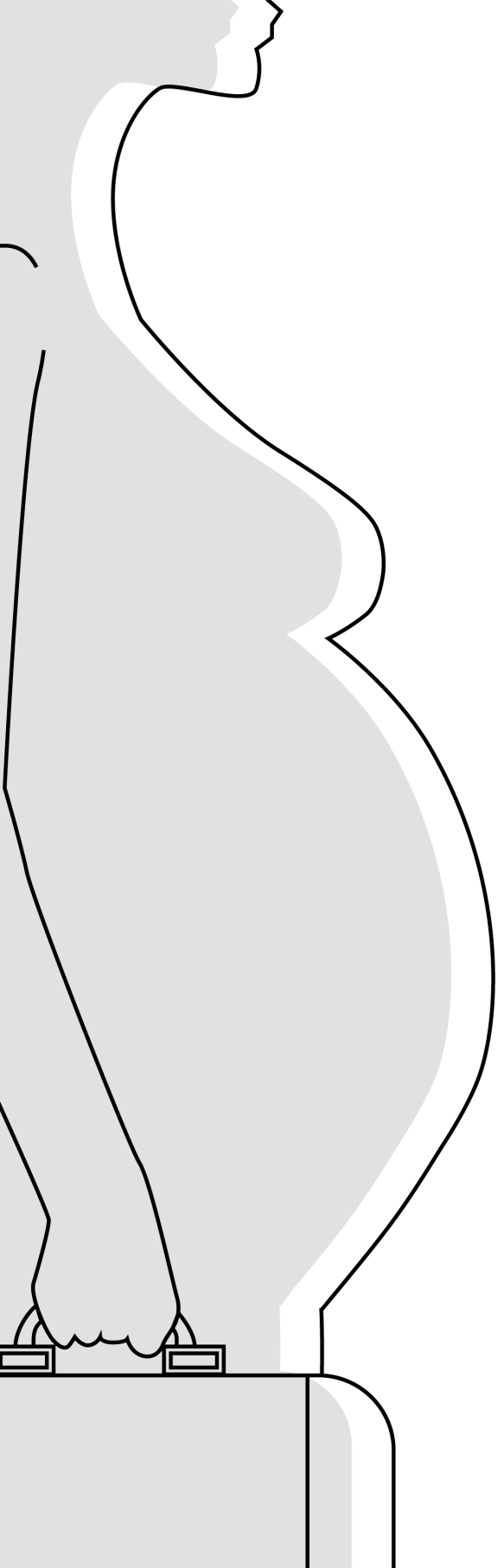
PART 2

OCCUPATIONAL EXPOSURE TO CHEMICALS



Chapter 2.1

Chemicals and time to pregnancy: A systematic review

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ABSTRACT

Background: Fertility problems are an important health issue, as 10-15% of couples have difficulties conceiving. Reproductive function is thought to be compromised by life style behaviours, but environmental contaminants and work-related factors are also thought to play a role. The objective of this review was to systematically summarise the available evidence concerning the influence of occupational exposure to chemicals on time to pregnancy (TTP).

Methods: A structured search on occupational exposure to chemicals and TTP was carried out in Pubmed and Embase. Studies were included if TTP was used as outcome measure and exposure to chemicals at job level was described. In total, 49 studies were included in this review.

Results: Studies varied widely in characterisation of exposure, hampering a meta-analytic approach across all studies. For lead strong indications for adverse effects on TTP were present, supporting the mandatory provisions for pregnant women being exposed to lead in many countries. These indications were also found for pesticide exposure, and one could argue that couples working in agriculture or horticultural trades must be informed about the risks of pesticide exposure. Epidemiologic evidence on other chemicals, such as organic solvents, and other metals remains equivocal, hampering clear counselling of couples who are trying to get pregnant.

Conclusion: Despite some uncertainties in the evidence base, it may still be prudent to advise against lead and pesticide exposure at the workplace for couples trying to conceive. This review also identifies several priorities for future studies in the field of occupational epidemiology.

INTRODUCTION

Fertility problems are an important health issue, as 10-15% of couples have difficulties conceiving a child and seek specialist fertility care at least once during their reproductive lifetime.[1] The ability of a couple to procreate is determined by the chance of conception leading to a live birth per menstrual cycle given unprotected intercourse, fecundability in demographic terms, and is influenced by various male and female factors. The distribution of individual couple probabilities is extremely heterogeneous, varying from a zero chance (sterile couples, who never become pregnant) to an estimated upper limit of 60% per menstrual cycle for 'super fertile' couples who conceive in the first month.[2,3] Hence, the time it takes to become pregnant since actively trying to conceive, time to pregnancy (TTP), is a measure of couple fertility. TTP as outcome measure has been extensively used in epidemiological studies aiming to identify the effects of, for example, adverse lifestyle or changes in fertility over time.[4-7]

Lifestyle factors related to postponement of motherhood,[8,9] smoking,[10,11] and alcohol or caffeine intake[12] may interfere with the reproductive system.[13] However, work-related and environmental risk factors may also reduce fertility.[14] Findings from contaminant residue analyses in human blood, follicular fluid and semen,[15,16] together with reports of a purported decline in semen quality,[17] led to the hypothesis that chemical contaminants may negatively affect the reproductive process causing reduced fertility and adverse pregnancy outcomes in the general population. However, there is an ongoing debate whether human fecundity is really declining in Western countries,[18] and it is of great importance to establish whether environmental chemicals adversely affect human reproduction, so that preventive measures, if needed, could be taken. A number of reviews have reported on associations between exposure to specific chemicals or groups of chemicals and TTP, and support the notion that environmental exposures may be hazardous for human fertility.[19-23] However, all of these reviews focussed on the effects of, mainly male, exposure to one specific chemical or a group of chemicals on fertility or reproductive function rather than focussing on the broader spectrum of chemical exposure and their effects on TTP in both men and women.

From an obstetric point of view, chemicals that influence TTP may subsequently influence pregnancy and birth outcomes, either directly or indirectly. Several studies showed that a prolonged TTP is associated with a greater risk of adverse pregnancy outcomes.[24-26] Exposure to chemicals during foetal development may increase the risk of adverse health consequences, including adverse birth outcomes, childhood morbidity, and adult disease and mortality.[27,28]

A comprehensive review of the literature concerning occupational exposure to chemicals in relation to TTP, supported by findings from animal studies, and observational studies on the influence of chemicals on other fertility outcomes such as semen quality, might improve the clinician's ability to counsel couples who are trying to conceive or women who have concerns about their pregnancy.[29] With this review, we aimed to summarise the evidence on occupational exposure to chemicals and TTP, and to describe exposure-response relationships in order to determine hazardous levels of exposure for prolonged TTP.

MATERIALS AND METHODS

Literature search

The first author (CS) conducted a systematic literature search on articles up to December 2010 in Pubmed and Embase using the following key words: toxic actions, environmental pollution, chemical, hazard, accident, occupational exposure, occupation, occupational diseases, work, worker, workplace, vocation, job, employment, industry, business, profession, trade, enterprise. These keywords were combined with key words used for TTP, fertility, fecundity, fecundability, subfertility, infertility, infertile, and time to pregnancy (TTP). In addition, a hand search was done to explore the references of articles retrieved. The complete search strategy is available on request.

Eligibility and selection

Articles were initially selected based on title and abstract according to the following inclusion criteria: (1) TTP was used as outcome measure in occupational or general populations, (2) a quantitative description of measures of exposure to chemical agents at the workplace or a description of a distinct exposure pattern at job level was presented, (3) the associations between work-related exposure and TTP were expressed in a quantitative measure, such as odds ratio (OR), relative risk (RR), or fecundability ratio (FR) or sufficient raw data were presented to calculate such measures of association and (4) the article was published in a peer reviewed scientific journal written in the English, German, French, or Dutch language.

The literature search identified 1412 articles in Pubmed and 1304 articles in Embase, resulting in a total of 2017 unique articles. The initial selection on title and subsequently on abstract was done by the first author (CS) and verified by the last author (AB) and resulted in a selection of 147 articles (Supplement 1). Subsequently, the second (EV) and last author (AB) independently made a further selection based on abstracts which resulted in 85 relevant articles (overlap between both authors was 83%). The selected full articles were then judged by two authors (CS, AB) based on the above-mentioned inclusion and exclusion criteria. Six articles were excluded due to TTP not being used as an outcome measure. Five articles were excluded because they were reviews and one study because it was based on preliminary results. No distinct pattern of occupational exposure was present in 21 articles. One article was excluded because no quantitative measure of association was reported, a second article because it was published in Spanish, and a third article because no full text was available. This resulted in a total of 49 relevant articles for this review.

Assessment of methodological quality

The quality of the epidemiological studies was assessed by two reviewers (CS and AB) using a standardised form based on seven items in a modified version of the guidelines for methodological quality assessment of the Dutch Cochrane Centre:[30]

1. Research hypothesis: prior to the study, the researchers should have formulated an hypothesis setting out the relation between exposure to chemicals in a particular profession and the possible effects on TTP;
2. Study population: the study groups should be clearly defined (exposed versus non-exposed), and at least age, sex and occupation should be described in detail;
3. Selection bias: any attempt to detect selection bias requires that the study groups' inclusion and exclusion criteria be clearly defined. It is important that the response at baseline should exceed 50%;
4. Exposure: exposure should be clearly defined. Details should also be provided of the instrument used to identify the determinant, and of when and under what circumstances this was done. This should be performed in the same way in each study group. Exposure assessment is done in the relevant time window (assessment during TTP period).
5. Outcome: the outcome itself and the criteria used to determine the outcome should be sufficiently clearly defined to enable the work to be reproduced by other researchers. The outcome should be determined using a valid measurement method. The outcome should be blind for exposure status.
6. Confounding: the analysis should be adjusted for confounders;
7. General opinion: assessment of the study's validity and applicability.

Each criterion was rated when applicable, with a score of 1 being sufficiently met, a score of 0 being not sufficiently met, and a question mark when the information was lacking to rate this item. The total quality score ranged from 0 to 7. The influence of quality score on the reported measures of associations was evaluated.

Data extraction

The data extraction on selected articles comprised the study setting, study population, study design, outcome(s), exposure assessment, confounders or effect modifiers, and effect estimates (with 95% confidence intervals). The data extraction for the study population included the following items: number of invited employees, eligibility criteria, participation, total number in the analyses and number of lost to follow up, if applicable. For characteristics of exposure the definition of magnitude, frequency or duration of exposure as well as the prevalence of exposure was extracted. In addition, it was ascertained whether the study addressed only maternal occupational exposure, paternal occupational exposure, or occupational exposure among both partners of the couple involved. Whenever possible, the measure of association was retrieved from the original article, together with the variables that were used for adjustment in the statistical analyses. If articles adjusted for relevant confounders and concluded that the confounders did not significantly influence the effect estimates, and therefore presented unadjusted estimates, the unadjusted estimates were presented in the Tables and adjustment for confounders was summarised as 'no significant adjustments'. When the measure of

association was not present, available raw data were used in a 2 x 2 Table to calculate the OR and 95% confidence interval as measure of association.

Data extraction was performed by one author according to a standardised format (CS) and extracted data were reviewed by the last author for consistency and completeness (AB). In case of doubt, data were discussed until agreement was reached.

Construction of plots

Supplement 2 represents a funnel plot to identify potential publications bias. Since most studies used FR as measure of association, the funnel was constructed for studies with a FR. When a job title was used as proxy for exposure, we used the FR for comparing TTP among workers in this job with workers in other jobs. When a direct measurement or comprehensive method of exposure assessment was used, we included the highest exposure category with the accompanying FR. Figure 1 provides an overview of the studies included in this review, where a division in male, female, and couples studies was based on occupational exposure to chemicals, and estimates were sorted by occupations or jobs studied, groups of chemicals, and specific chemicals, in order of specificity of the exposure. Risk estimates of individual studies were used to calculate pooled estimates when studies had comparable outcome measures (FRs or ORs), and when studies were considered sufficiently comparable with respect to exposure parameters, which resulted in pooled estimates for studies using a standardised job title as proxy for exposure. We used the computer program epi-sheet to calculate pooled FRs, applying a fixed model assumption by default.[31]

RESULTS

In total, 49 relevant articles were included in this review in accordance with the flow chart depicted in Supplement 1. The studies were divided into three separate categories, based on occupational exposure to chemicals, namely studies concerning men, studies concerning women, and studies addressing couples, and corresponding Tables were constructed. Table 1 describes the core results of all occupational studies concerning men and is subdivided into four groups of chemicals: pesticides, heavy metals, (organic) solvents, and miscellaneous chemicals. In a similar way, Table 2 presents all occupational studies concerning women, and Table 3 reports on studies concerning couples. The full version of Tables 1-3 is available in Supplement 3-5. We also made a division into three types of exposure groups: 1) articles concerning a specific occupation, 2) articles focussing on various groups of chemicals, and 3) articles reporting on a specific chemical.

Figure 1 summarises all studies that estimated a fecundability ratio (FR) (37 out of 49 studies), and provides an overview by exposure status (maternal, paternal, and parental occupational exposure to chemicals), gender, and type of study. In the first group of studies, addressing a specific occupation in relation to TTP, 23 studies reported 28 FRs, of which ten FRs (36%) were

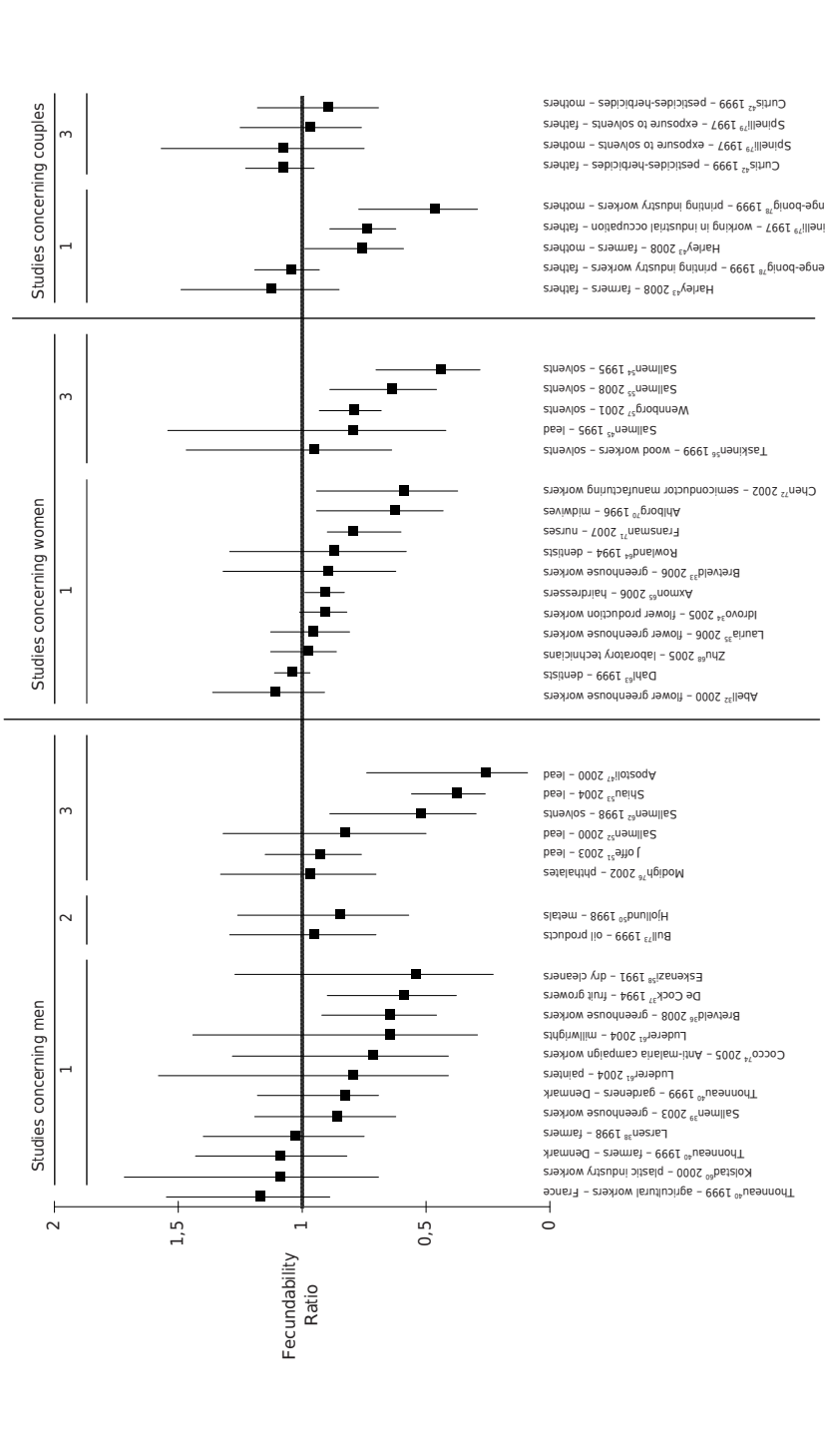


FIGURE 1. Associations between specific occupations (1), groups of chemicals (2), and specific chemicals (3) with TTP, expressed as a fecundability ratio, stratified by paternal, maternal and parental exposure

1) specific occupations for which we took either the specific occupation or, if an exposure index was used, the highest exposure category, 2) groups of substances, 3) specific substances.

statistically significantly reduced. In the second group, addressing exposure to a group of chemicals, two studies reported a FR, and both showed no statistically significant association. Group 3 addressed exposure to one specific chemical and 13 studies reported 15 FRs, of which six (40%) were statistically significantly reduced. One study reported both exposure based on occupation and exposure to a specific chemical and was included in both groups. In all studies among men, 17 studies reported 20 FRs, of which five (25%) were statistically significantly reduced. In 16 studies among women, 16 FRs were reported, of which seven (44%) were statistically significantly reduced.

We summarised all studies that used an OR to quantify the relation between chemicals exposure and TTP in Figure 2, using the same division as in Figure 1.

Occupations most studied in relation to fecundability were occupations with exposure to pesticides and heavy metals. Nearly all studies performed a cross-sectional analysis, either as part of a cross-sectional study design or as part of an analysis of the baseline within a cohort study. Various methods were used to assess occupational exposure to chemicals: 20 studies used a questionnaire, ten studies interviewed participants, one study solely used direct measurements, eight studies used both a questionnaire and direct measurements, while ten studies used a combination of a questionnaire, expert judgement, an interview, or (in)direct measurements.

Quality of evidence

Supplement 6 presents the methodological quality assessment of the studies included. The two reviewers initially agreed on 72% of the studies (307 out of 343 items); all initial disagreements were resolved in a consensus meeting. The quality scores ranged from 3 to 7. A crude measure of exposure and an unclear definition of assessment of TTP were the most prevalent shortcomings in quality. We observed that studies with a low quality score, namely a score of 3, less often reported statistically significant associations than high quality studies with a score of 7, 25% and 50%, respectively. The median number of participants in the various studies was 541 (range: 40-7079). Most of the studies focussed on TTP as a continuous outcome enabling analyses with Cox Proportional Hazards models, producing FRs.

Publication bias

The funnel plot in Supplement 2 clearly suggests publication bias, whereby smaller studies with a decreased FR were more likely to be published since almost no small studies published negative findings.

Occupational exposure to pesticides

In total, 13 studies addressed occupational exposure to pesticides in relation to TTP. Four studies were performed among women, and FRs ranged from 0.64 to 1.11,[32-35] while only two studies found statistically significant effects.[32,34] Six studies were performed among men, with FRs ranging from 0.43 to 1.18,[36-40] and Petrelli and Figa-Talamanca presented ORs of a TTP more

TABLE 1. Core associations between occupational exposure to chemicals and time to pregnancy (males)

First Author	Population	Exposure	Results		Important confounders
Pesticides					
Bretveld, 2008 Netherlands[36]	694 greenhouse workers versus 613 shopkeepers and market stallholders	Working in greenhouse	FR 1.12	1.00-1.26	Smoking, alcohol, education
		Working in greenhouse, only primigravidous	FR 0.65	0.46-0.92	
Petrelli, 2001 Italy[41]	127 greenhouse workers versus 173 administrative workers	Working as greenhouse worker, low exposure	OR 1.6	0.8-3.1	Age, smoking
		(1-100 hrs application per year) (TTP>6 months)			
		Working as greenhouse workers, high exposure	OR 2.4	1.2-5.1	
		(>100 hrs application per year) (TTP>6 months)			
Sallmen, 2003 Finland[39]	522 greenhouse workers	Work at greenhouses or gardens	FDR 0.86	0.62-1.19	Age, smoking, alcohol, last contraceptive method
		No efficient protection, low exposure	FDR 0.77	0.46-1.29	
		No efficient protection, medium exposure	FDR 0.92	0.45-1.88	
		No efficient protection, high exposure	FDR 0.67	0.33-1.35	
		Not efficiently protected, low-moderate exposure, primiparous	FDR 0.43	0.22-0.81	
		Not efficiently protected, high exposure, primiparous	FDR 0.31	0.10-0.99	
		Protected efficiently, primiparous	FDR 0.59	0.34-1.01	
De Cock, 1994 Netherlands[37]	91 pregnancies of fruit growers	Spraying velocity <1.5 hectares	FR 0.53	0.33-0.85	Gravidity
		Crop area < 10 hectares	FR 0.59	0.38-0.90	
		Spraying days <15 days/yr	FR 0.48	0.25-0.90	
Larsen, 1998 Denmark[38]	616 farmers	Traditional farmers (spraying pesticides) vs organic farmers	FR 1.03	0.75-1.40	Age, smoking, recent use contraceptives, parity
		Traditional farmers (spraying pesticides) vs traditional farmers (not spraying pesticides)	FR 1.18	0.83-1.66	
		Exposure index group 2 vs 1 (based on number of hectares sprayed, type of crop, way of spraying)	FR 0.83	0.58-1.17	
		Exposure index group 3 versus 1	FR 0.92	0.64-1.31	

TABLE 1 (continued). Core associations between occupational exposure to chemicals and time to pregnancy (males)

First Author	Population	Exposure	Results		Important confounders
Thonneau, 1999 France/ Denmark[40]	362 French agricultural workers, 449 Danish farmers and 121 Danish greenhouse workers	France: working in agriculture and use of pesticides	FR 1.17	0.89-1.55	Age, parity, smoking, use contraceptives
		Denmark: conventional vs organic farmers	FR 1.09	0.82-1.43	
		Denmark: gardeners vs organic famers	FR 0.83	0.69-1.18	
Heavy metals					
Apostoli, 2000 Italy[47]	251 workers from 10 lead related factories versus 45 controls from non-lead related factories	Blood lead level 20-29 µg/dl	FR 0.91	0.50-1.68	Age
		Blood lead level 30-39 µg/dl	FR 1.13	0.57-2.24	
		Blood lead level 40+ µg/dl	FR 0.26	0.09-0.74	
Bonde, 1990 Denmark[48]	53 cases of delayed conception among welders/non-welders compared to 208 age-matched referents and 563 birth referents	Exposed as welder vs. age matched non-exposed (TTP>12 months)	OR 1.85	0.84-4.08	Smoking, alcohol, parity, underlying diseases
		Exposed as welder vs. birth referents non-exposed (TTP>12 months)	OR 2.02	1.02-4.00	
Greene, 2010 Mongolia[49]	61 pregnancies of leather tannery workers versus 145 pregnancies from bread factory personnel	Working as tannery worker (TTP > 6 months)	OR 1.1	0.3-4.2	Age
		Working as tannery worker (TTP>12 months)	OR 2.8	0.9-9.0	
Hjollund, 1998 Denmark[50]	406 metal workers and welders	Welders vs. nonwelding metal workers	fOR 0.85	0.57-1.26	Age, use contraceptives, diseases, smoking, alcohol
		Welders vs. nonmetal workers	fOR 1.12	0.83-1.53	
Joffe, 2003 Belgium/ Finland/ Italy/ England[51]	868 workers from lead related companies versus 236 workers in non-lead related companies	Blood lead level 20-29 µg/dl (compared to internal control group)	HR 0.96	0.77-1.19	Age, smoking, parity
		Blood lead level 30-39 µg/dl	HR 0.88	0.70-1.10	
		Blood lead level 40+ µg/dl	HR 0.93	0.76-1.15	
		Cumulative values (µg/dl/year) <120 (internal control group)	HR 0.94	0.73-1.18	
		Cumulative values (µg/dl/year) 120-220	HR 0.97	0.78-1.22	
		Cumulative values (µg/dl/year) 220-420	HR 0.71	0.56-0.89	
		Cumulative values (µg/dl/year) 420+	HR 1.04	0.83-1.31	

TABLE 1 (continued). Core associations between occupational exposure to chemicals and time to pregnancy (males)

First Author	Population	Exposure	Results		Important confounders
Sallmen, 2000 Finland[52]	502 workers monitored for lead exposure	Estimated blood lead level 0.5-0.9 µmol/l	FR 0.92	0.73-1.16	Age, use contraceptives
		Estimated blood lead level 1.0-1.4 µmol/l	FR 0.89	0.66-1.20	
		Estimated blood lead level 1.5-1.8 µmol/l	FR 0.58	0.33-0.96	
		Estimated blood lead level >1.9 µmol/l	FR 0.83	0.50-1.32	
Shiau, 2004 Taiwan[53]	280 pregnancies of workers in a lead battery plant	Lead exposure <20 µg/dl (all pregnancies)	FR 0.91	0.61-1.35	Age
		Lead exposure 20-29 µg/dl	FR 0.71	0.46-1.09	
		Lead exposure 30-39 µg/dl	FR 0.50	0.34-0.74	
		Lead exposure >40 µg/dl	FR 0.38	0.26-0.56	
(Organic) solvents					
Eskenazi, 1991 USA[58]	14 dry cleaners exposed to perchloroethylene versus 26 laundry workers	Working as laundry worker versus working as dry cleaner	fRR 0.54	0.23-1.27	Ethnicity, smoking
		PCE level (natural log)	fRR 0.94	0.85-1.04	
		Exposure index	fRR 0.90	0.78-1.03	
Hooiveld, 2006 Netherlands[59]	472 painters versus 462 carpenters	Working as painter vs carpenter (TTP>12 months, all)	OR 1.1	0.7-1.9	Age, smoking, alcohol
		Painters low exposure (0.17-0.38 mg/m^3) versus carpenters	OR 1.2	0.5-2.5	
		Painters medium exposure (0.38-1.02 mg/m^3) versus carpenters	OR 1.1	0.5-2.2	
		Painters high exposure (1.03-4.66 mg/m^3) versus carpenters	OR 1.1	0.5-2.7	
Kolstad, 2000 Denmark/ Italy/ Netherlands[60]	220 plastic industry workers exposed to styrene versus 382 non-exposed internal controls	Low exposure to styrene	FR 0.68	0.48-0.97	Age, use contraceptives, smoking
		Medium exposure to styrene	FR 0.70	0.48-1.04	
		High exposure to styrene	FR 1.09	0.69-1.72	
Luderer, 2004 USA[61]	32 painters versus 40 carpenters and 25 millwrights	Working as millwright versus carpenter	FR 0.65	0.29-1.44	Age, smoking
		Working as painter versus carpenter	FR 0.80	0.41-1.58	
Sallmen, 1998 Finland[62]	282 workers monitored for solvent exposure	Low/intermediate solvent exposure, primiparous	FDR 0.62	0.34-1.13	Age, smoking
		High/frequent solvent exposure, primiparous	FDR 0.52	0.30-0.89	

TABLE 1 (continued). Core associations between occupational exposure to chemicals and time to pregnancy (males)

First Author	Population	Exposure	Results		Important confounders
Other					
Bull, 1999 Norway[73]	182 offshore mechanics, operators, drilling personnel, car mechanics exposed to oil products versus 68 non-exposed carpenters and joiners	All exposed workers to oil products versus carpenter	FR 0.95	0.70-1.29	Age, parity, coffee
		Working as car mechanic versus carpenter	FR 1.00	0.68-1.45	
		Working as mechanic offshore versus carpenter	FR 0.85	0.53-1.39	
		Working as drilling personnel versus carpenter	FR 0.93	0.66-1.32	
		Working as operator versus carpenter	FR 1.08	0.72-1.61	
Cocco, 2005 Italy[74]	49 anti-malaria campaign workers exposed to DDT/DDE versus 24 non-exposed referents	DDT applicators	FR 0.72	0.41-1.28	Age
		Men exposed to DDT	FR 0.84	0.50-1.43	
Figa-Talamanca, 2000 Italy[75]	153 mint workers exposed to a mixture of chemicals, metal fumes and solvents	Working as technical versus administrative (TTP>6 months)	OR 2.58	0.51-13.09	Age, smoking, alcohol, education
		Working as stamper versus administrative	OR 3.03	0.40-23.04	
		All manual workers versus administrative	OR 2.57	0.52-12.65	
Modigh, 2002 Sweden[76]	326 pregnancies of workers in plants producing DEHP	Low exposure to DEHP (<0.1mg/m^3)	FR 1.07	0.84-1.35	Age
		High exposure to DEHP (>0.1 mg/m^3)	FR 0.97	0.70-1.33	
Ford, 2002 England[7]	4808 pregnancies of workers in various occupations exposed to mixtures of chemicals	Working in printing or related trades (TTP>6 months)	OR 1.86	1.21-2.94	Age, ethnicity, education, BMI, smoking, alcohol
		Working in printing or related trades (TTP>12 months)	OR 1.96	1.13-3.39	

Abbreviations: FR, fecundability ratio; OR, Odds Ratio; FDR, fecundability density ratio; fOR, fecundability odds ratio; HR, hazard ratio; fRR, fecundability rate ratio, CS, cross-sectional study; C, cohort study; CC, case-control study.

TABLE 2. Core associations between occupational exposure to chemicals and time to pregnancy (females)

First Author	Population	Exposure	Results		Important confounders
Pesticides					
Abell, 2000 Denmark[32]	253 flower greenhouse workers versus 239 referents	Working in flower greenhouse	FR 1.11	0.90-1.36	Age, smoking, parity, education, use contraceptives
		High vs low exposure within greenhouse workers	FR 0.64	0.45-0.90	
Idrovo, 2005 Colombia[34]	2085 flower production workers	Working in flower greenhouse (yes)	fOR 0.91	0.82-1.01	Age
		Working in flower greenhouse >2 years	fOR 0.73	0.63-0.84	
Lauria, 2006 Italy[35]	713 pregnancies of flower greenhouse workers Working in agriculture		FR 0.96	0.81-1.13	Age, parity, smoking, alcohol
Bretveld, 2006 Netherlands[33]	398 greenhouse workers versus 524 shopkeepers and cleaners	Working in greenhouse	FR 1.11	0.96-1.29	Age, smoking., parity
		Working in agriculture (full time workers and first pregnancies)	FR 0.90	0.62-1.32	
Heavy metals					
Sallmen, 1995 Finland[45]	121 metal-, chemical-, and graphic workers monitored for exposure to lead	Blood lead level <0.5 µmol/l	IDR 0.93	0.56-1.57	Age, parity, alcohol
		Blood lead level 0.5-0.9 µmol/l	IDR 0.84	0.48-1.45	
		Blood lead level >1.0 µmol/l	IDR 0.80	0.42-1.54	
Wulff, 1999 Sweden[46]	703 women working in a smelter or living near a smelter	Working as smelter (TTP>12 months)	OR 0.91	0.48-1.90	Age, parity, smoking, alcohol, education
		Living near smelter (TTP>12 months)	OR 0.82	0.37-1.82	
(Organic) solvents					
Sallmen, 1995 Finland[54]	197 workers monitored for solvent exposure	Solvent exposure category low	IDR 0.74	0.49-1.11	Age, alcohol
		Solvent exposure category high	IDR 0.44	0.28-0.70	
Sallmen, 2008 Finland[55]	197 shoe manufacturing workers versus 209 workers in food units and storehouses	Solvent exposure category low	FDR 0.55	0.40-0.74	Use contraceptives, smoking, alcohol
		Solvent exposure category high	FDR 0.70	0.52-0.94	
		Solvent exposure category low (primiparous)	FDR 0.56	0.39-0.79	
		Solvent exposure category high (primiparous)	FDR 0.64	0.46-0.89	

TABLE 2 (continued). Core associations between occupational exposure to chemicals and time to pregnancy (females)

First Author	Population	Exposure	Results		Important confounders
Taskinen, 1999 Finland[56]	602 wood workers exposed to solvents and formaldehyde	Low exposure to formaldehyde (0.07ppm)	FDR 1.09	0.86-1.37	Smoking, alcohol
		Medium exposure to formaldehyde (0.14ppm)	FDR 0.96	0.72-1.26	
		High exposure to formaldehyde (0.33ppm)	FDR 0.64	0.43-0.92	
Wennborg, 2001, Sweden[57]	560 laboratory workers	Solvents exposure	FR 0.79	0.68-0.93	Age, diseases
		Benzene exposure	FR 0.75	0.44-1.29	
		Acetone exposure	FR 0.72	0.53-0.97	
		Chloroform exposure	FR 0.96	0.75-1.22	
Other					
Dahl, 1999 Norway[63]	558 dentists exposed to amalgam and chloroform versus 450 high school teachers	Practicing dentistry first pregnancy	FR 1.00	0.99-1.01	Age, smoking, diseases
		Placing amalgam >100/wk	FR 1.04	0.97-1.11	
		Placing chloroform	FR 1.06	0.95-1.10	
Rowland, 1994\ USA[64]	407 dentists exposed to mercury vapour	1-14 amalgams per week	FDR 1.33	1.03-1.72	Use contraceptives, age, ethnicity, smoking, diseases
		15-29 amalgams per week	FDR 1.25	0.97-1.63	
		30-59 amalgams per week	FDR 0.90	0.68-1.19	
		60+ amalgams per week	FDR 0.87	0.58-1.29	
		>30 amalgams per week, 5-8 poor hygiene factors	FDR 0.63	0.42-0.96	
Axmon, 2006 Sweden[65]	1418 hairdressers versus 1578 referents general population	Working as hairdresser	FR 0.91	0.83-0.99	No sign adjustments
Kersemaekers, 1997 Netherlands[66]	1854 hairdressers versus 1332 salesclerks	Working as hairdresser 1986-1988 (TTP>12months)	OR 1.5	0.8-2.8	No sign adjustments
		Working as hairdresser 1991-1993	OR 1.2	0.8-1.6	
Peretz, 2009 USA[67]	450 cosmetologists versus 511 non-cosmetologists	Working as cosmetologist (TTP>12 months)	OR 0.82	0.57-1.17	Age, ethnicity, education, smoking, alcohol

TABLE 2 (continued). Core associations between occupational exposure to chemicals and time to pregnancy (females)

First Author	Population	Exposure	Results		Important confounders
Zhu, 2005 Denmark[68]	829 laboratory technicians versus 6250 teachers	Laboratory workers vs. teachers	FR 0.94	0.86-1.02	Age, parity, smoking
		Laboratory workers vs. teachers, primigravidae	FR 0.98	0.86-1.13	
Ahlborg, 1996 Sweden[70]	972 midwives exposed to nitrous oxide	1-10 nitrous oxide deliveries per month	FR 1.18	0.98-1.41	Age, diseases, use contraceptives
		11-20 nitrous oxide deliveries per month	FR 1.05	0.86-1.28	
		21-20 nitrous oxide deliveries per month	FR 1.19	0.89-1.59	
		30+ nitrous oxide deliveries per month	FR 0.63	0.43-0.94	
Fransman, 2007 Netherlands[71]	1519 nurses exposed to antineoplastic drugs	Nurses with low exposure (<0.20 µg/wk)	HR 0.9	0.7-1.0	Age, parity, smoking, alcohol
		Nurses with medium exposure (0.21-0.74 µg/wk)	HR 1.0	0.8-1.2	
		Nurses with high exposure (>0.74 µg/wk)	HR 0.8	0.6-0.9	
Schaumberg, 1989 Denmark[69]	2557 pregnancies of pharmacists	Working in production (TTP>12 months all)	OR 1.03	0.7-1.5	Parity, smoking, alcohol
		Working in dispensary	OR 0.70	0.5-1.0	
		Working in identification/control	OR 0.55	0.2-1.3	
		Working in bottling	OR 0.36	0.1-2.9	
		Working in packing	OR 0.62	0.1-2.8	
Chen, 2002 Taiwan[72]	188 semiconductor manufacturing workers exposed to ethylene glycol ethers versus 104 pregnancies of non-fabrication workers	Self reported use of glycol ethers	FR 0.59	0.37-0.94	Age, parity, use contraceptives
		Working in thin film area	FR 1.85	1.10-3.12	
		Working in photolithography area	FR 0.77	0.45-1.32	
		Working in diffusion area	FR 1.68	0.95-2.98	
		Working in etching area	FR 1.44	0.94-2.21	
		Working in testing area	FR 1.25	0.61-2.56	

Abbreviations: FR, fecundability ratio; OR, Odds Ratio; FDR, fecundability density ratio; fOR, fecundability odds ratio; HR, hazard ratio; IDR, incidence density ratio, CS, cross-sectional study; C, cohort study; CC, case-control study. No sign adjustments: adjustments did not significantly influence the effect estimates, and therefore unadjusted estimates were presented.

TABLE 3. Core associations between occupational exposure to chemicals and time to pregnancy (couples)

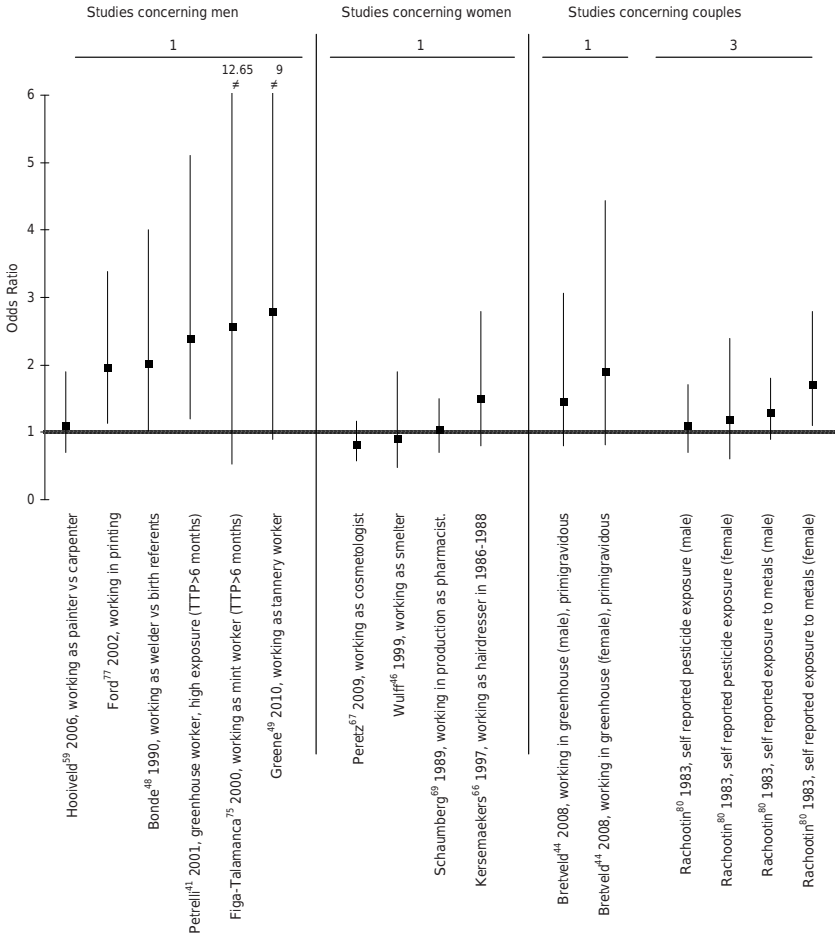
First Author	Population	Exposure	Results		Important confounders
Pesticides					
Bretveld, 2008 Netherlands[44]	101 female and 957 male greenhouse workers versus 1408 referents	Working in greenhouse (female) (TTP>12months, all), primigravidous	OR 1.90	0.81-4.44	Age, smoking
		Working in greenhouse (male), primigravidous	OR 1.46	0.79-3.06	
Harley, 2008 USA[43]	402 couples working as farmers	Mother agriculture	fOR 0.76	0.59-0.99	Age, use contraceptives, diseases
		Father agriculture	fOR 1.13	0.85-1.49	
		Pesticides used in home	fOR 0.64	0.43-0.94	
		Level of p.p'-DDT (female)	fOR 0.96	0.79-1.18	
		Level of p.p'-DDE (female)	fOR 0.91	0.68-1.22	
		Level of o.p'-DDT (female)	fOR 0.98	0.79-1.21	
Curtis, 1999 USA[42]	1048 couples working as farmers	Pesticides use, women's activity, herbicides	FR 0.90	0.69-1.18	Age, use contraceptives, smoking
		Pesticides use, women's activity, insecticides	FR 1.02	0.76-1.37	
		Pesticide use, men's activity, herbicides	FR 1.08	0.95-1.23	
		Pesticide use, men's activity, insecticides	FR 1.01	0.87-1.17	

TABLE 3 (continued). Core associations between occupational exposure to chemicals and time to pregnancy (couples)

First Author	Population	Exposure	Results		Important confounders
Other					
Plenge-Bonig, 1999, Germany[78]	269 pregnancies from printing industry workers exposed to toluene	Working in printing industry (men)	FR 1.05	0.93-1.19	Age, smoking, parity
		Working in printing industry (women)	FR 0.47	0.29-0.77	
Rachootin, 1983 Denmark[80]	436 couples with an infertility problem versus 3728 control couples with a healthy born child	Pesticides (men) (TTP>12 months, all)	OR 1.1	0.7-1.7	Age, education, parity, smoking, alcohol, use contraceptives
		Pesticides (women)	OR 1.2	0.6-2.4	
		Lead, mercury and cadmium (men)	OR 1.3	0.9-1.8	
		Lead, mercury and cadmium (women)	OR 1.7	1.1-2.8	
		Plastic manufacturing (men)	OR 1.7	1.0-2.9	
		Plastic manufacturing (women)	OR 2.1	1.2-3.9	
		Welding of other materials (men)	OR 1.8	1.1-1.8	
		Welding of other metals (women)	OR 1.5	1.1-5.1	
Spinelli, 1997 Italy[79]	622 couples studied for exposure to various chemicals	Exposure to solvents (women)	FR 1.08	0.75-1.57	Age, parity, smoking, alcohol, work
		Working in industrial occupation (men)	FR 0.74	0.62-0.89	
		Exposure to solvents (men)	FR 0.97	0.76-1.25	

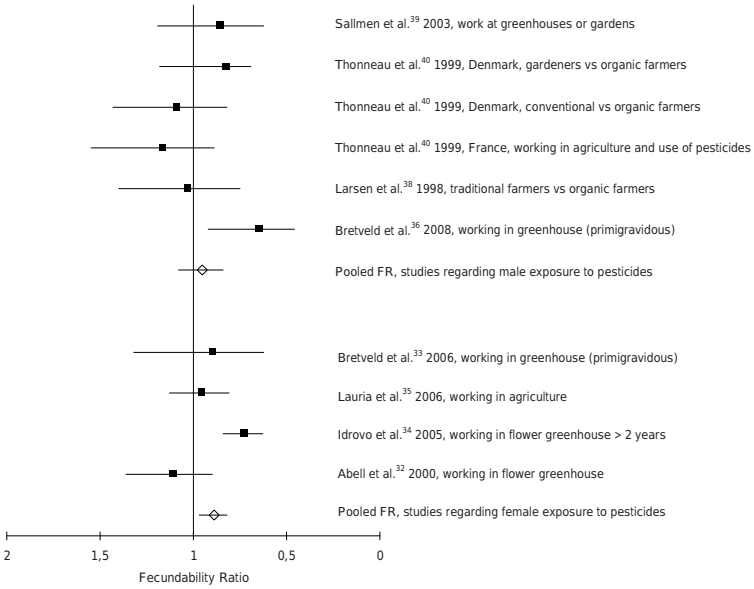
Abbreviations: FR, fecundability ratio; OR, Odds Ratio; fOR, fecundability odds ratio, CS, cross-sectional study; C, cohort study; CC, case-control study.

FIGURE 2. Associations between specific occupations (1), groups of chemicals (2), and specific chemicals (3) with TTP, expressed as Odds Ratio (TTP>12 months), stratified by paternal, maternal, and parental exposure



than six months ranging between 1.6 to 2.4.[41] Four of these studies found statistically significant effects.[36,37,39,41] Three studies addressed both women and men, with FRs ranging from 0.64 to 1.13[42,43] and ORs for a TTP of 12 months or longer ranging from 0.65 to 1.90.[44] Among these 13 studies, seven studies used a job title as proxy for exposure,[33-35,36,38,40,44] two studies used a more comprehensive method, such as an exposure index,[37,41] three studies combined job title with another method of exposure assessment,[32,39,43] and one study relied on self-reported exposure to pesticides.[42] Eight studies with job title as proxy for exposure to pesticides reported a FR, as shown in Figure 3. The pooled estimates were FR 0.95 (95%CI 0.84-1.08) for men and FR 0.89 (95%CI 0.82-0.97) for women. Figure 4 shows the forest plot summarising female and male studies using a more comprehensive method of exposure assessment that reproduced a FR. These four studies showed most FRs as being statistically significantly reduced.

FIGURE 3. Forest plot summarising studies concerning male and female pesticide exposure (with job title as proxy for exposure) and fecundability



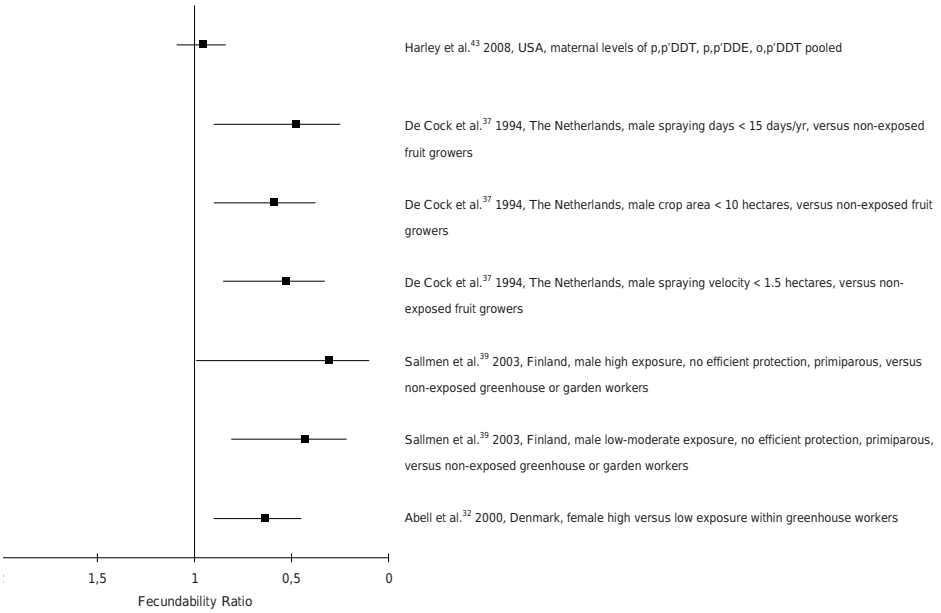
Occupational exposure to heavy metals

Nine studies reported exposure to heavy metals in relation to TTP, of which two studies were performed among women,[45,46] and seven studies among men.[47-53] In the two studies among women, the FRs ranged from 0.80 to 0.93 for lead exposure and from 0.82 to 0.91 for exposure to a mixture of metals. In men, four studies reported that lead exposure (measured as blood lead levels) reduced fecundability,[47,51-53] two studies observed increased risks of TTP longer than 12 months among welders and tannery workers (OR 2.02; 95%CI 1.02-4.00, OR 2.8; 95%CI 0.9-9.0, respectively),[48,49] and another study among welders found FRs ranging from 0.85 to 1.12.[50] Figure 5 depicts the exposure-response relationship between different levels of blood lead values and FRs, showing a clear trend of increasing blood lead levels with decreasing FRs.

Occupational exposure to (organic) solvents

Nine studies addressed occupational exposure to (organic) solvents in relation to TTP. Four studies were conducted among women,[54-57] with FRs ranging from 0.44 to 1.09, for different solvents. Five studies were conducted among men,[58-62] with FRs ratios ranging from 0.52 to 1.09. Three studies performed (in)direct measurements, and found FRs ranging from 0.52 to 1.09,[58,60,62] and one study was based on interviews, with FRs raging from 0.65-0.80.[61] A large study by Hooiveld et al., combining questionnaires and indirect measurements, presented ORs around unity.[59]

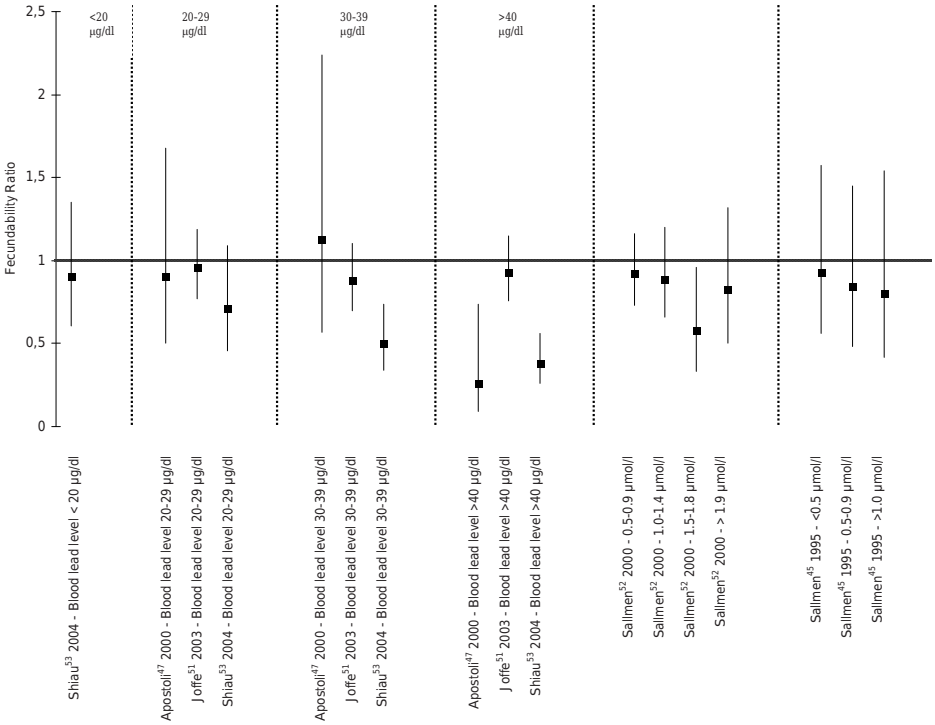
FIGURE 4. Forest plot summarising studies concerning male and female pesticide exposure (more comprehensive measure of exposure) and fecundability



Occupational exposure to 'other' chemicals

In both women and men, several studies addressed occupational exposure to a mixture of chemicals or a specific chemical, most often in one specific occupation. In women, occupations investigated were dentists,[63,64] hairdressers,[65,66] cosmetologists,[67] laboratory technicians,[68] pharmacists,[69] health care workers, such as nurses and midwives,[70,71] and semiconductor manufacturing workers.[72] For dentists, hairdressers, midwives working with nitrous oxide, nurses working with antineoplastic drugs, and semiconductor manufacturing workers in thin film area, some indications were found for a prolonged TTP.[64,65,70-72] In men, occupations studied were offshore mechanics, operators, drilling personnel, car mechanics,[73] antimalaria campaign workers,[74] mint workers,[75] and workers in plants producing di(2-ethylhexyl)phthalate (DEHP),[76] while one study addressed various occupations.[77] In studies on couples, printing industry workers were studied,[78] as well as occupations in industry with self-reported exposure to solvents and fumes.[79] In a case-control study on couples with or without infertility treatment, several occupations and work situations were studied[80] of which several seemed to be risk factors for subfertility.

FIGURE 5. Forest plot summarising studies concerning dose response relations between blood lead levels and time to pregnancy



DISCUSSION

From this review we can conclude that there are strong indications that certain occupational exposures, such as pesticides and lead, adversely influence male and female fertility. These associations were primarily observed in studies with a detailed exposure assessment strategy, whereby different levels of exposure could be distinguished. In studies with job title as proxy for exposure these findings could not be corroborated, since only moderate or non-statistically significant associations were reported in most. For other chemicals, such as exposure to (organic) solvents and specific occupations, the evidence of effects on time to pregnancy (TTP) is less clear, hampering clear advice for couples who intend to become pregnant.

Lack of exposure-response associations, weak exposure assessments, and the large heterogeneity in exposure characterisation across studies were the primary limitations to the hypothesis that occupational chemical exposure adversely affects human reproduction. The evaluation of possible publication bias indicates that this is a serious threat, since smaller studies tended to get published more often when reporting a significantly longer TTP with occupational exposure to chemicals.

Time to pregnancy studies: weight of evidence and biological plausibility with regard to chemical exposure

Couple fertility depends on complex biological processes such as the production of sperm and oocytes, the fertilisation process, the implantation of the embryo, the transition from embryo to foetus, and the growth of the foetus into a matured child. Although little is known about chemical agents interacting with these processes, exposure to chemicals may adversely affect this chain of reproductive events at any phase but the sensitivity may vary. With regard to female gametes, we speculate that oocytes may be most vulnerable to toxicants, chemotherapy or radiation during the foetal period when they are formed and multiply in both ovaries when a maximum number of some seven million oocytes are created. Thereafter, oocytes are surrounded by a protective layer of granulosa cells and will remain inactive until adulthood, and eventually only a few oocytes will mature and can be fertilised to become embryos.[81] Damage to oocytes during foetal development, may become manifest as reduced fertility. But also toxic exposure later in life, for instance through occupational exposure may induce cytotoxic harm to the oocytes and subsequently subfertility. Adult men produce millions of sperm cells every day, and we hypothesised that the fastly dividing sperm producing epithelium of the testis may be vulnerable to chemical exposures, not only during the foetal period when it is established, but throughout life. This may result in suboptimal sperm DNA integrity and semen quality and consequently, in reduced fertility.[82] To our knowledge, it is currently unknown, whether occupational exposure to chemicals affects male or female reproduction equally, or whether males or females have a different susceptibility for chemicals.

Existing literature

Several reviews summarised the literature regarding occupational exposure and fertility. However, to our knowledge, no previous reviews specifically focussed on occupational chemical exposure and TTP as a measure of fertility. Until now, three reviews assessed the influence of pesticides on male and/or female fertility.[19,21,22] They conclude that although the results of the studies are often equivocal, there are indications for an association between pesticide exposure and prolonged TTP. The majority of studies included in these reviews are also included in our study, but a few studies failed to fulfil our criteria, as they did not provide TTP but some other measure of fertility. Since we had several studies regarding pesticide exposure and TTP, we carried out a pooled analysis, combining effect estimates from studies that used job title as proxy for exposure. This meta-analysis showed no reduced fecundability ratios (FRs) (see Figure 3). However, several studies with a detailed exposure assessment strategy, characterised as an exposure index of (in)direct measurements, clearly suggest that pesticide exposure may increase the risk of a prolonged TTP (see Figure 4). Thus, it seems that using a job title as proxy for pesticide exposure is not a sensitive enough measure for assessing exposure, since it will introduce non-differential misclassification and consequently bias of the measure of association towards unity. In addition, the assessment of exposure to pesticides was not able

to pinpoint to the role of specific pesticides as often a cocktail of various pesticides was used in the occupational groups studied. Three reviews summarised the literature on occupational exposure to lead and the evidence is quite consistent, showing that lead exposure reduces fertility and prolongs TTP.[23,83,84] From our review, we can also conclude that there are strong indications that lead exposure prolongs TTP, since higher blood lead levels were often associated with a longer TTP.

We also identified some more generic reviews on occupation and reproductive function, not only focussing on chemical exposure, but also on other potentially harmful working conditions, such as physical load, and psychological stress. From these studies, it is clear that the number of substances potentially hazardous to male reproduction is large, but that for few agents only the evidence is unequivocal.[20,85] Figa-Talamanca et al. reported several associations between exposure to metals, solvents, and pesticides and male reproductive effects.[86] These reviews present the picture that the reproductive function of males is vulnerable to many different environmental and occupational agents. However, only a few of these external agents have so far been identified with certainty, most often limited to men intoxicated by a specific chemical or among workers with high exposures. Several studies have identified alterations in fertility, but the results are difficult to replicate in other settings with different patterns of exposure.

Recall bias

Ascertainment of TTP requires only a few simple questions (such as: How many months did it take to become pregnant?). Refusal to answer these questions is rare, as this question is readily accepted in a wide range of cultures.[87] Validation studies have shown that self-reports on TTP give an accurate representation of the true TTP distribution,[88-90] even with recall up to 20 years.[91] Men can also provide valid information, generating the same distributions and analytical results as women in the same study population.[9,92] Since most studies included in this review collected data on occupational characteristics, such as a job title, work activities, or obtained direct measurements from workers, and did not rely on self-reported exposures, we think that recall bias does not seem to be a major issue in the studies included in this systematic review.

Exposure assessment

Exposure assessment, the study of the distribution and determinants of substances or factors affecting human health, is an important area in occupational epidemiology. Traditionally, exposure in the workplace tends to be higher than in the general environment whereas the duration of exposure is generally shorter. Quantification of the association between exposure and adverse human health effects requires the use of exposure estimates, which are valid, precise and biologically relevant for the critical exposure period, and show a range of exposure levels in the population under study.[93] In this review, most studies relied on exposure assessment through questionnaires, but also other techniques, such as expert judgement, direct

measurements in body fluids or tissues, and comprehensive measures such as an integrated exposure index were used. This heterogeneity in exposure assessment made it impossible to perform a meta-analysis across all studies. However, we noticed that studies with direct measurements or comprehensive measures more often showed an association between exposure and TTP. This was also illustrated by the fact that studies with lower quality less often reported statistically significant findings compared to high quality studies. An important reason for a lower quality was a poor characterisation of exposure. Use of expert judgements, for example through a job-exposure-matrix, is easier and cheaper than using direct measurements, and will resolve some of the problems encountered using self-assessment or a job title as proxy for exposure. Exposure assessment with a job-exposure-matrix is done independently from the health outcome and blinded to participants, both aspects that will avoid information bias. If sufficient information on work tasks and type of business is available, non-differential misclassification of exposure can be reduced. A more objective way to assess exposure is through measurements, which is generally expensive and time-consuming, especially in community-based studies. This systematic review shows that improvements in exposure assessment in studies on occupational risk factors for TTP is urgently required, whereby a combination of different methods may be the way forward.[94] Whenever possible, studies should be designed to provide effect estimates for chemical mixtures and take into account the combined effects of chemicals.[95] Exposure timing also needs more consideration in future studies. Assessment of occupational exposure during the TTP period is essential, and possible changes in these periods must be addressed to prevent misclassification.

Background exposure to various chemicals through diet and environment may occur. However, it is unlikely that background exposure will contribute substantially to the exposure patterns in selected occupational populations.[93] In community-based studies the high prevalence of background exposure will most likely not be associated with occupational exposure with a much lower prevalence. Thus, background exposure has probably not confounded the reported associations between occupational chemical exposure and TTP in this review.

Study design and confounding

A prospective study design has definite advantages, but since this is very time-consuming, retrospective studies on TTP are far more common. Most of the studies included in this review were cross-sectional studies, and information on occupational exposures often was collected retrospectively. Therefore, interpretation of the results from these studies may be hampered by biases related to recruitment, treatment, accidental pregnancies, degree of planning and persistency of trying, social background, sexual behaviour, female age, and non-response.[4,6,96] In order to reduce various sources of bias, the analysis should focus on first pregnancies, as it will avoid pregnancy planning issues in which past-pregnancy experiences are taken into account.[97] Only a few studies included in this review have provided a separate analysis on

primigravidous couples, and thus, future studies should focus more on first pregnancies, since this will provide more valid effect estimates.

Confounding is a major concern within observational studies, since these pose a serious threat to the internal validity. We tried to address confounding and selection bias in the included studies by a comprehensive quality assessment based on the guidelines for quality assessment of the Dutch Cochrane Centre. Age, educational level, body mass index, and smoking are factors associated with TTP, and these factors may act as confounders if they are also associated with chemical exposure at the workplace. Future studies need to adjust for age by default, since age will influence the reproductive abilities of both men and women. Furthermore, TTP is a measure of couples' fecundability, and if only men or women are studied, confounding by partner could occur. Therefore, studies should focus more on couples instead of only focussing on men or women.

Publication bias

We addressed publication bias with a funnel plot. Studies that report statistically significant associations are more likely to be published and this may bias reviews towards concluding that associations truly exist. It appeared that smaller studies more often showed lower FRs, thus a prolonged TTP and it seemed that smaller studies reporting null effects were published less than what would be expected based on the funnel plot. We may conclude that the results of this review, to some extent, may suffer from publication bias. However, we must note that the smaller studies that more often showed an association with TTP also used more comprehensive methods or direct measurement for assessing exposure. Since these measurements are expensive, it is obvious that these studies have smaller sample sizes.

Limitations

This systematic review has several limitations. Although we searched through all the references of the articles selected, it cannot be ruled out that relevant publications have been missed. The second limitation is that the majority of studies found were of cross-sectional design and, as a consequence, causality cannot be established. Third, the large heterogeneity in the articles retrieved, made it impossible to perform a meta-analysis across all studies.

CONCLUSION

In total, 49 studies reported associations between occupational exposure to chemicals and TTP. On the basis of this systematic review, the evidence from studies regarding exposure to pesticides and lead is suggestive for adverse effects on human reproduction, in particular a prolonged TTP. For other chemical exposures and specific occupations, the evidence is less

clear, not justifying mandatory restrictions on occupational activities of couples who try to become pregnant.

In an effort to achieve more specificity and replication in this field, the next wave of studies investigating the effects of occupational chemical exposure on fertility should attempt to 1) evaluate the effects of specific individual chemicals, as well as mixtures, 2) use biomonitoring methods to quantify the compounds in human fluids such as blood and urine, enabling dose response studies, 3) focus on couples, since TTP is a measure of couples' fecundability, specifically for occupational exposures to chemicals in the workplace among partners in order to provide more insight into the separate effects of maternal and paternal exposure on TTP, and 4) ensure adequate control for confounders. Further human studies are necessary to clarify both the effects of current occupational exposures on reproductive health and the physiologic mechanisms underlying these effects.

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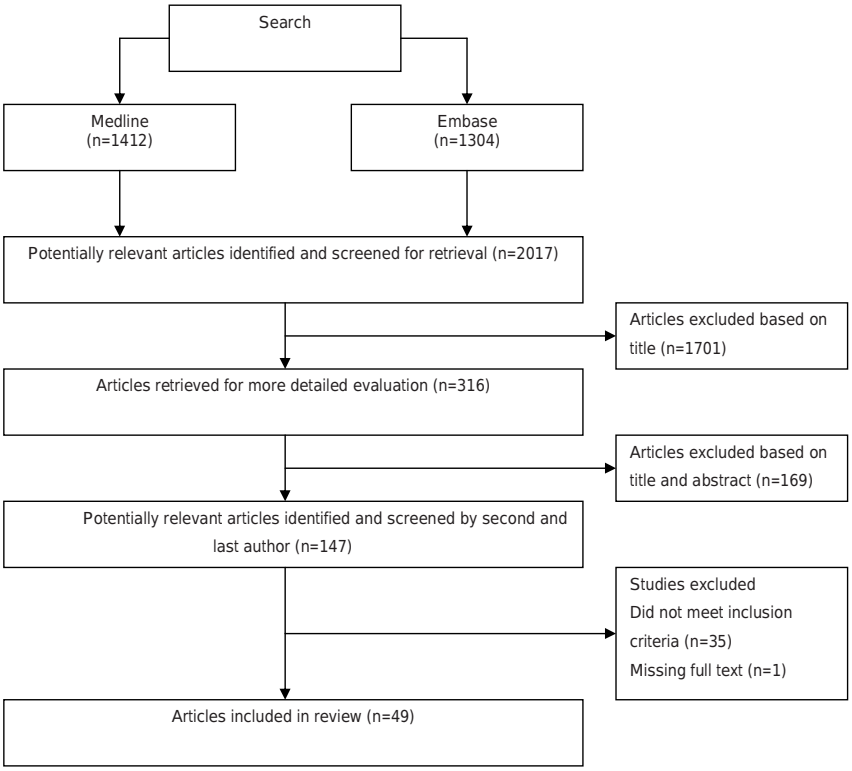
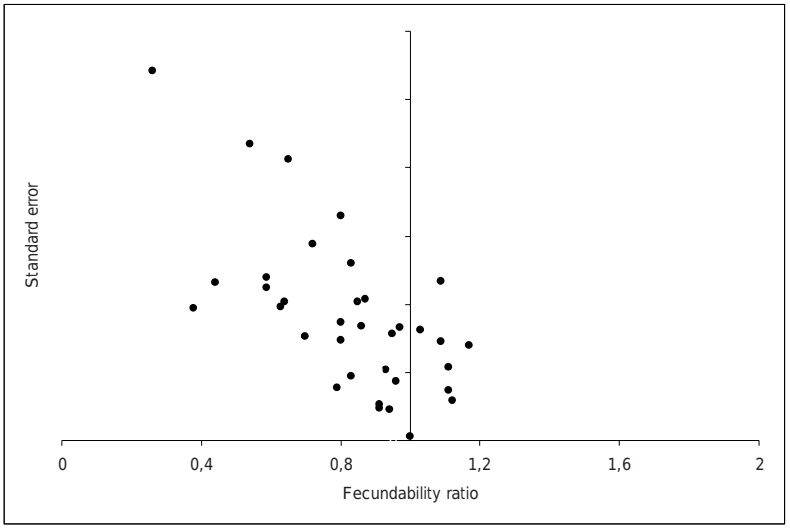
SUPPLEMENT 1. Flow chart of the process for selection of relevant articles



SUPPLEMENT 2. Funnel plot for detection of publication bias in studies addressing occupational exposure to chemicals among men and women in relation to time to pregnancy



SUPPLEMENT 3. Associations between occupational exposure to chemicals and time to pregnancy (males)

First Author	Occupation	Chemical	Design	n	Exposure		Results		Important confounders
Pesticides									
Bretveld, 2008 Netherlands[36]	Greenhouse workers	Pesticides	CS	1307	Questionnaire	Working in greenhouse	FR 1.12	1.00-1.26	Smoking, alcohol, education
						Working in greenhouse, only primigravidous	FR 0.65	0.46-0.92	
Petrelli, 2001 Italy[41]	Greenhouse workers	Pesticides	CS	300	Questionnaire	Working as greenhouse worker, low exposure (1-100 hrs application per year) (TTP>6 months)	OR 1.6	0.8-3.1	Age, smoking
						Working as greenhouse workers, high exposure (>100 hrs application per year) (TTP>6 months)	OR 2.4	1.2-5.1	
Sallmen, 2003 Finland[39]	Greenhouse workers	Pesticides	CS	522	Questionnaire and expert judgment	Work at greenhouses or gardens	FDR 0.86	0.62-1.19	Age, smoking, alcohol, last contraceptive method
						No efficient protection, low exposure	FDR 0.77	0.46-1.29	
						No efficient protection, medium exposure	FDR 0.92	0.45-1.88	
						No efficient protection, high exposure	FDR 0.67	0.33-1.35	
						Protected efficiently, low exposure	FDR 0.82	0.51-1.34	
						Protected efficiently, medium exposure	FDR 1.15	0.63-2.11	
						Protected efficiently, high exposure	FDR 0.94	0.58-1.52	
						Not efficiently protected, low-moderate exposure, working at least 4 months	FDR 0.93	0.54-1.60	
						Not efficiently protected, high exposure, working at least 4 months	FDR 0.73	0.31-1.71	
						Protected efficiently, working at least 4 months	FDR 1.07	0.66-1.71	
						Not efficiently protected, low-moderate exposure, primiparous	FDR 0.43	0.22-0.81	
						Not efficiently protected, high exposure, primiparous	FDR 0.31	0.10-0.99	
						Protected efficiently, primiparous	FDR 0.59	0.34-1.01	
De Cock, 1994 Netherlands[37]	Fruit growers	Pesticides	CS	91	Questionnaire	Spraying velocity <1.5 hectares	FR 0.53	0.33-0.85	Gravidity
						Crop area < 10 hectares	FR 0.59	0.38-0.90	
						Spraying days <15 days/yr	FR 0.48	0.25-0.90	

SUPPLEMENT 3 (continued). Associations between occupational exposure to chemicals and time to pregnancy (males)

First Author	Occupation	Chemical	Design	n	Exposure		Results		Important confounders
Larsen, 1998 Denmark[38]	Farmers	Pesticides	CS	616	Interview	Traditional farmers (spraying pesticides) vs organic farmers	FR 1.03	0.75-1.40	Age, smoking, recent use contraceptives, parity
						Traditional farmers (spraying pesticides) vs traditional farmers (not spraying pesticides)	FR 1.18	0.83-1.66	
						Exposure index group 2 vs 1 (based on number of hectares sprayed, type of crop, way of spraying)	FR 0.83	0.58-1.17	
						Exposure index group 3 versus 1	FR 0.92	0.64-1.31	
Thonneau, 1999\ France/ Denmark[40]	Agricultural workers	Pesticides	CS	932	Questionnaire or interview	France: working in agriculture and use of pesticides	FR 1.17	0.89-1.55	Age, parity, smoking, recent use contraceptives
						Denmark: conventional vs organic farmers	FR 1.09	0.82-1.43	
						Denmark: gardeners vs organic famers	FR 0.83	0.69-1.18	
Heavy metals									
Apostoli, 2000 Italy[47]		Lead	CS	296	Direct measurement	Blood lead level 20-29 µg/dl	FR 0.91	0.50-1.68	Age
						Blood lead level 30-39 µg/dl	FR 1.13	0.57-2.24	
						Blood lead level 40+ µg/dl	FR 0.26	0.09-0.74	
Bonde, 1990 Denmark[48]	Welders	Metals	CS	824	Questionnaire	Exposed as welder vs. age matched non-exposed (TTP>12 months)	OR 1.85	0.84-4.08	Smoking, alcohol, parity, underlying diseases
						Exposed as welder vs. birth referents non-exposed (TTP>12 months)	OR 2.02	1.02-4.00	
Greene, 2010 Mongolia[49]	Leather tannery workers	Metals	CS	206	Interview	Working as tannery worker (TTP > 6 months)	OR 1.1	0.3-4.2	Age
						Working as tannery worker (TTP>12 months)	OR 2.8	0.9-9.0	
Hjollund, 1998 Denmark[50]	Metal workers	Metals	C	406	Questionnaire and direct measurement	Welders vs. nonwelding metal workers Welders vs. nonmetal workers	fOR 0.85 fOR 1.12	0.57-1.26 0.83-1.53	Age, contraceptives, smoking, alcohol

SUPPLEMENT 3 (continued). Associations between occupational exposure to chemicals and time to pregnancy (males)

First Author	Occupation	Chemical	Design	n	Exposure	Results		Important confounders
Joffe, 2003 Belgium/ Finland/ Italy/ England[51]		Lead	CS	1104	Expert judgementand direct measurements	Blood lead level 20-29 µg/dl (compared to internal control group, all)	HR 0.96	0.77-1.19	Age, smoking, parity
						Blood lead level 30-39 µg/dl	HR 0.88	0.70-1.10	
						Blood lead level 40+ µg/dl	HR 0.93	0.76-1.15	
						Duration of exposure 0-4 years (compared to internal control group, all)	HR 0.92	0.76-1.12	
						Duration of exposure 5-9 years	HR 0.92	0.75-1.13	
						Duration of exposure 10-14 years	HR 0.78	0.62-1.01	
						Duration of exposure 15+ years	HR 1.31	0.96-1.77	
						Cumulative values (µg/dl/year) <120 (compared to internal control group, all)	HR 0.94	0.73-1.18	
						Cumulative values (µg/dl/year) 120-220	HR 0.97	0.78-1.22	
						Cumulative values (µg/dl/year) 220-420	HR 0.71	0.56-0.89	
						Cumulative values (µg/dl/year) 420+	HR 1.04	0.83-1.31	
Sallmen, 2000 Finland[52]		Lead	CS	502	Questionnaire and direct measurement	Estimated blood lead level 0.5-0.9 µmol/l	FR 0.92	0.73-1.16	Age, use contraceptives
						Estimated blood lead level 1.0-1.4 µmol/l	FR 0.89	0.66-1.20	
						Estimated blood lead level 1.5-1.8 µmol/l	FR 0.58	0.33-0.96	
						Estimated blood lead level >1.9 µmol/l	FR 0.83	0.50-1.32	
Shiau, 2004 Taiwan[53]	Workers in lead battery plant	Lead	CS	280	Interview and direct measurement	Lead exposure <20 µg/dl (all pregnancies)	FR 0.91	0.61-1.35	Age
						Lead exposure 20-29 µg/dl	FR 0.71	0.46-1.09	
						Lead exposure 30-39 µg/dl	FR 0.50	0.34-0.74	
						Lead exposure >40 µg/dl	FR 0.38	0.26-0.56	
						Lead exposure <20 µg/dl (first pregnancies)	FR 0.88	0.50-1.53	
						Lead exposure 20-29 µg/dl	FR 0.62	0.33-1.14	
						Lead exposure 30-39 µg/dl	FR 0.52	0.27-0.99	
						Lead exposure >40 µg/dl	FR 0.48	0.23-0.78	

SUPPLEMENT 3 (continued). Associations between occupational exposure to chemicals and time to pregnancy (males)

First Author	Occupation	Chemical	Design	n	Exposure	Results			Important confounders
(Organic) solvents									
Eskenazi, 1991 USA[58]	Dry cleaners	Perchloro-ethylene	CS	40	Interview and direct measurements	Working as laundry worker	fRR 0.54	0.23-1.27	Ethnicity, smoking
						PCE level (natural log)	fRR 0.94	0.85-1.04	
						Exposure index	fRR 0.90	0.78-1.03	
Hooiveld, 2006 Netherlands[59]	Painters	Solvents	CS	934	Questionnaire	Working as painter vs carpenter (TTP>12 months, all)	OR 1.1	0.7-1.9	Age, smoking, alcohol
						Painters low exposure (0.17-0.38 mg/m^3) versus carpenters	OR 1.2	0.5-2.5	
						Painters medium exposure (0.38-1.02 mg/m^3) versus carpenters	OR 1.1	0.5-2.2	
						Painters high exposure (1.03-4.66 mg/m^3) versus carpenters	OR 1.1	0.5-2.7	
Kolstad, 2000 Denmark/ Italy/ Netherlands[60]	Plastic industry workers	Styrene	CS	602	Questionnaire and direct measurement	Low exposure to styrene	FR 0.68	0.48-0.97	Age, use contraceptives, smoking
						Medium exposure to styrene	FR 0.70	0.48-1.04	
						High exposure to styrene	FR 1.09	0.69-1.72	
						(all countries)			
Luderer, 2004 USA[61]	Painters and millwrights	Solvents	CS	97	Interview	Working as millwright versus carpenter	FR 0.65	0.29-1.44	Age, smoking
						Working as painter versus carpenter	FR 0.80	0.41-1.58	
Sallmen, 1998 Finland[62]		Solvents	CS	282	Questionnaire and direct measurement	Low/moderate solvent exposure	FDR 0.74	0.51-1.06	Age, smoking
						High/frequent solvent exposure	FDR 0.80	0.57-1.11	
						Low/intermediate solvent exposure, primiparous	FDR 0.62	0.34-1.13	
						High/frequent solvent exposure, primiparous	FDR 0.52	0.30-0.89	

SUPPLEMENT 3 (continued). Associations between occupational exposure to chemicals and time to pregnancy (males)

First Author	Occupation	Chemical	Design	n	Exposure		Results		Important confounders
Other									
Bull, 1999 Norway[73]	Offshore mechanics, operators, drilling personnel, car mechanics	Oil products	CS	250	Questionnaire	All exposed workers to oil products versus carpenter	FR 0.95	0.70-1.29	Age, parity, coffee
						Working as car mechanic versus carpenter	FR 1.00	0.68-1.45	
						Working as mechanic offshore versus carpenter	FR 0.85	0.53-1.39	
						Working as drilling personnel versus carpenter	FR 0.93	0.66-1.32	
						Working as operator versus carpenter	FR 1.08	0.72-1.61	
Cocco, 2005 Italy[74]	Anti-malaria campaign workers	DDT and DDE	CS	73	Interview	DDT applicators	FR 0.72	0.41-1.28	Age
						Men exposed to DDT	FR 0.84	0.50-1.43	
Figa-Talamanca, 2000 Italy[75]	Mint workers	Mixture of chemicals, metal fumes and solvents	CS	153	Questionnaire	Working as technical versus administrative	OR 2.58	0.51-13.09	Age, smoking, alcohol, education
						Working as stamper versus administrative	OR 3.03	0.40-23.04	
						Working as founder versus administrative	OR 2.19	0.34-14.20	
						All manual workers versus administrative	OR 2.57	0.52-12.65	
						(TTP>6 months)			
Modigh, 2002 Sweden[76]	Workers in plants producing DEHP	Phthalates	CS	326	Interview and direct measurement	Low exposure to DEHP (<0.1mg/m^3)	FR 1.07	0.84-1.35	Age
						High exposure to DEHP (>0.1 mg/m^3)	FR 0.97	0.70-1.33	
Ford, 2002 England[77]	Various occupations	Mixtures	CS	4808	Questionnaire	Working in printing or related trades (TTP>6 months)	OR 1.86	1.21-2.94	Age, ethnicity, education, BMI, smoking, alcohol
						Working in printing or related trades (TTP>12 months)	OR 1.96	1.13-3.39	

Abbreviations: FR, fecundability ratio; OR, Odds Ratio; FDR, fecundability density ratio; fOR, fecundability odds ratio; HR, hazard ratio; fRR, fecundability rate ratio, CS, cross-sectional study; C, cohort study; CC, case-control study.

SUPPLEMENT 4. Associations between occupational exposure to chemicals and time to pregnancy (females)

First Author	Occupation	Chemical	Design	n	Exposure		Results		Adjustments
Pesticides									
Abell, 2000 Denmark[32]	Flower greenhouse workers	Pesticides	CS	492	Interview	Working in flower greenhouse	FR 1.11	0.90-1.36	Age, smoking, parity, education, use contraceptives
						High vs low exposure within greenhouse workers	FR 0.64	0.45-0.90	
Idrovo, 2005 Colombia[34]	Flower production workers	Pesticides	CS	2085	Interview	Working in flower greenhouse (yes)	fOR 0.91	0.82-1.01	Age
						Working in flower greenhouse < 2 years	fOR 0.86	0.75-0.98	
						Working in flower greenhouse >2 years	fOR 0.73	0.63-0.84	
Lauria, 2006 Italy[35]	Flower greenhouse workers	Pesticides	CS	713	Interview	Working in agriculture	FR 0.96	0.81-1.13	Age, parity, smoking, alcohol
Bretveld, 2006 Netherlands[33]	Greenhouse workers	Pesticides	CS	922	Questionnaire	Working in greenhouse	FR 1.11	0.96-1.29	Age, smoking, parity
						Working in agriculture (full time workers)	FR 0.89	0.67-1.19	
						Working in agriculture (full time workers and first pregnancies)	FR 0.90	0.62-1.32	
Heavy metals									
Sallmen, 1995 Finland[45]	Metal-, chemical-, and graphic workers	Lead	CS	121	Questionnaire Direct Measurement	Blood lead level <0.5 µmol/l	IDR 0.93	0.56-1.57	Age, parity, alcohol
						Blood lead level 0.5-0.9 µmol/l	IDR 0.84	0.48-1.45	
						Blood lead level >1.0 µmol/l	IDR 0.80	0.42-1.54	
Wulff, 1999 Sweden[46]	Smelters	Mixture of metals	CS	703	Questionnaire	Working as smelter (TTP>12 months)	OR 0.91	0.48-1.90	Age, parity, smoking, alcohol, education
						Living near smelter (TTP>12 months)	OR 0.82	0.37-1.82	
(Organic) solvents									
Sallmen, 1995 Finland[54]		Solvents	CS	197	Questionnaire Direct measurement	Solvent exposure category low	IDR 0.74	0.49-1.11	Age, alcohol
						Solvent exposure category high	IDR 0.44	0.28-0.70	
						Both among women employed at beginning TTP period			

SUPPLEMENT 4 (continued). Associations between occupational exposure to chemicals and time to pregnancy (females)

First Author	Occupation	Chemical	Design	n	Exposure	Results			Adjustments
Sallmen, 2008 Finland[55]	Shoe manufacturing workers	Solvents	CS	406	Indirect measurements	Solvent exposure category low	FDR 0.55	0.40-0.74	Use contraceptives, smoking, alcohol
						Solvent exposure category high	FDR 0.70	0.52-0.94	
						Solvent exposure category low (primiparous)	FDR 0.56	0.39-0.79	
						Solvent exposure category high (primiparous)	FDR 0.64	0.46-0.89	
Taskinen, 1999, Finland[56]	Wood workers	Solvents Formalde-hyde	CS	602	Questionnaire Expert judgment	Low exposure to formaldehyde (0.07ppm)	FDR 1.09	0.86-1.37	Smoking, alcohol
						Medium exposure to formaldehyde (0.14ppm)	FDR 0.96	0.72-1.26	
						High exposure to formaldehyde (0.33ppm)	FDR 0.64	0.43-0.92	
						Low exposure to solvents (8.7% of OEL)	FDR 0.93	0.71-1.21	
						Medium exposure to solvents (17.0% of OEL)	FDR 0.91	0.68-1.21	
						High exposure to solvents (31.9% of OEL)	FDR 0.95	0.64-1.47	
Wennborg, 2001, Sweden[57]	Laboratory workers	Solvents	CS	560	Questionnaire	Solvents exposure	FR 0.79	0.68-0.93	Age, diseases
						Benzene exposure	FR 0.75	0.44-1.29	
						Acetone exposure	FR 0.72	0.53-0.97	
						Chloroform exposure	FR 0.96	0.75-1.22	
						Diethylether exposure	FR 1.06	0.77-1.46	
						Phenol exposure	FR 0.90	0.70-1.16	

SUPPLEMENT 4 (continued). Associations between occupational exposure to chemicals and time to pregnancy (females)

First Author	Occupation	Chemical	Design	n	Exposure	Results			Adjustments
Other									
Dahl, 1999 Norway[63]	Dentists	Amalgam and chloroform	CS	1008	Questionnaire	Practicing dentistry first pregnancy	FR 1.00	0.99-1.01	Age, smoking, diseases
						Practicing dentistry >10 years first pregnancy	FR 1.03	1.00-1.07	
						Placing amalgam >100/wk	FR 1.04	0.97-1.11	
						Placing chloroform	FR 1.06	0.95-1.10	
Rowland, 1994 USA[64]	Dentists	Mercury vapour	CS	407	Questionnaire	1-14 amalgams per week	FDR 1.33	1.03-1.72	Use contraceptives, age, ethnicity, smoking, diseases
						15-29 amalgams per week	FDR 1.25	0.97-1.63	
						30-59 amalgams per week	FDR 0.90	0.68-1.19	
						60+ amalgams per week	FDR 0.87	0.58-1.29	
						1-14 amalgams per week, 5-8 poor hygiene factors	FDR 1.53	1.03-2.25	
						15-29 amalgams per week, 5-8 poor hygiene factors	FDR 1.14	0.73-1.77	
						>30 amalgams per week, 5-8 poor hygiene factors	FDR 0.63	0.42-0.96	
Axmon, 2006 Sweden[65]	Hairdressers	Mixture of chemicals	CS	2996	Questionnaire	Working as hairdresser	FR 0.91	0.83-0.99	No sign adjustments
Kersemaekers, 1997, Netherlands[66]	Hairdressers	Mixture of chemicals	CS	3186	Questionnaire	Working as hairdresser 1986-1988 (TTP>12 months)	OR 1.5	0.8-2.8	No sign adjustments
						Working as hairdresser 1991-1993	OR 1.2	0.8-1.6	
Peretz, 2009 USA[67]	Cosmetologists	Mixture	CS	961	Questionnaire	Working as cosmetologist (TTP>12 months)	OR 0.82	0.57-1.17	Age, ethnicity, education, smoking, alcohol

SUPPLEMENT 4 (continued). Associations between occupational exposure to chemicals and time to pregnancy (females)

First Author	Occupation	Chemical	Design	n	Exposure		Results		Adjustments
Zhu, 2005 Denmark[68]	Laboratory technicians	Mixture	CS	7079	Questionnaire and JEM	Laboratory workers vs. teachers	FR 0.94	0.86-1.02	Age, parity, smoking
						Laboratory workers vs. teachers, primigravidae	FR 0.98	0.86-1.13	
Ahlborg, 1996 Sweden[70]	Midwives	Nitrous oxide	CS	972	Questionnaire	1-10 nitrous oxide deliveries per month	FR 1.18	0.98-1.41	Age, diseases, use contraceptives
						11-20 nitrous oxide deliveries per month	FR 1.05	0.86-1.28	
						21-20 nitrous oxide deliveries per month	FR 1.19	0.89-1.59	
						30+ nitrous oxide deliveries per month	FR 0.63	0.43-0.94	
Fransman, 2007 Netherlands[71]	Nurses	Antineo-plastic drugs	CS	1519	Questionnaire and direct measurements	Nurses with low exposure (<0.20 µg/wk)	HR 0.9	0.7-1.0	Age, parity, smoking, alcohol
						Nurses with medium exposure (0.21-0.74 µg/wk)	HR 1.0	0.8-1.2	
						Nurses with high exposure (>0.74 µg/wk)	HR 0.8	0.6-0.9	
Schaumberg, 1989, Denmark[69]	Pharmacists	Mixture	CS	2557	Questionnaire	Working in production (TTP>12 months all)	OR 1.03	0.7-1.5	Parity, smoking, alcohol
						Working in dispensary	OR 0.70	0.5-1.0	
						Working in identification/control	OR 0.55	0.2-1.3	
						Working in bottling	OR 0.36	0.1-2.9	
						Working in packing	OR 0.62	0.1-2.8	
Chen, 2002 Taiwan[72]	Semiconductor manufacturing workers	Ethylene glycol ethers	CS	292	Interview	Self reported use of glycol ethers	FR 0.59	0.37-0.94	Age, parity, use contraceptives
						Working in thin film area	FR 1.85	1.10-3.12	
						Working in photolithography area	FR 0.77	0.45-1.32	
						Working in diffusion area	FR 1.68	0.95-2.98	
						Working in etching area	FR 1.44	0.94-2.21	
						Working in testing area	FR 1.25	0.61-2.56	

Abbreviations: FR, fecundability ratio; OR, Odds Ratio; FDR, fecundability density ratio; fOR, fecundability odds ratio; HR, hazard ratio; IDR, incidence density ratio, CS, cross-sectional study; C, cohort study; CC, case-control study. No sign adjustments: adjustments did not significantly influence the effect estimates, and therefore unadjusted estimates were presented.

SUPPLEMENT 5. Associations between occupational exposure to chemicals and time to pregnancy (couples)

First Author	Occupation	Chemical	Design	n	Exposure		Results		Adjustments
Pesticides									
Bretveld, 2008 Netherlands[44]	Greenhouse workers	Pesticides	CS	2466	Questionnaire	Working in greenhouse (female) (TTP>12 months, all)	OR 1.15	0.61-2.17	Age, smoking
						Working in greenhouse (male)	OR 0.65	0.49-0.94	
						Working in greenhouse (female), primigravidous	OR 1.90	0.81-4.44	
						Working in greenhouse (male), primigravidous	OR 1.46	0.79-3.06	
Harley, 2008 USA[43]	Farmers	DDT/DDE Pesticides	CS	402	Questionnaire Direct measurement	Mother agriculture	fOR 0.76	0.59-0.99	Age, use contraceptives, diseases
						Father agriculture	fOR 1.13	0.85-1.49	
						Pesticides used in home	fOR 0.64	0.43-0.94	
						Lives <200 ft from agricultural field	fOR 0.69	0.48-0.99	
						Level of p,p'-DDT (female)	fOR 0.96	0.79-1.18	
						Level of p,p'-DDE (female)	fOR 0.91	0.68-1.22	
						Level of o,p'-DDT (female)	fOR 0.98	0.79-1.21	
Curtis, 1999 USA[42]	Farmers	Pesticides	C	1048	Questionnaire	Pesticides use, women's activity, herbicides	FR 0.90	0.69-1.18	Age, use contraceptives, smoking
						Pesticides use, women's activity, insecticides	FR 1.02	0.76-1.37	
						Pesticide use, men's activity, herbicides	FR 1.08	0.95-1.23	
						Pesticide use, men's activity, insecticides	FR 1.01	0.87-1.17	
Other									
Plenge-Bonig, 1999 Germany[78]	Printing industry workers	Toluene	CS	269	Interview	Working in printing industry (men)	FR 1.05	0.93-1.19	Age, smoking, parity
						Working in printing industry (women)	FR 0.47	0.29-0.77	

SUPPLEMENT 5 (continued). Associations between occupational exposure to chemicals and time to pregnancy (couples)

First Author	Occupation	Chemical	Design	n	Exposure	Results			Adjustments
Rachootin, 1983 Denmark[80]		Various chemicals	CC	4164	Questionnaire Self-reports	Anesthetics (men) (TTP>12 months all)	OR 0.9	0.4-2.0	Age, education, parity, smoking, alcohol, use contraceptives
						Anesthetics (women)	OR 1.6	1.0-2.6	
						Pesticides (men)	OR 1.1	0.7-1.7	
						Pesticides (women)	OR 1.2	0.6-2.4	
						Weed killers (men)	OR 0.9	0.6-1.4	
						Weed killers (women)	OR 1.0	0.5-2.3	
						Degreasers (men)	OR 1.1	0.9-1.4	
						Degreasers (women)	OR 0.7	0.5-1.1	
						Lacquer, paint, glue (men)	OR 1.0	0.8-1.3	
						Lacquer, paint, glue (women)	OR 1.0	0.7-1.4	
						Cutting lubricating oils (men)	OR 1.2	0.9-1.5	
						Cutting lubricating oils (women)	OR 1.2	0.5-2.6	
						Dry cleaning chemicals (men)	OR 1.2	0.7-1.9	
						Dry cleaning chemicals (women)	OR 1.6	0.9-2.9	
						Textile dyes (men)	OR 2.2	1.1-4.2	
						Textile dyes (women)	OR 1.0	0.4-2.2	
						Lead, mercury and cadmium (men)	OR 1.3	0.9-1.8	
						Lead, mercury and cadmium (women)	OR 1.7	1.1-2.8	
						Plastic manufacturing (men)	OR 1.7	1.0-2.9	
						Plastic manufacturing (women)	OR 2.1	1.2-3.9	
						Welding of other materials (men)	OR 1.8	1.1-1.8	
						Welding of other metals (women)	OR 1.5	1.1-5.1	
Spinelli, 1997, Italy[79]		Various chemicals	CS	622	Interview	Exposure to solvents (women)	FR 1.08	0.75-1.57	Age, parity, smoking , alcohol, work factors
						Working in industrial occupation (men)	FR 0.74	0.62-0.89	
						Exposure to solvents (men)	FR 0.97	0.76-1.25	
						Exposure to fumes (men)	FR 0.78	0.78-1.00	

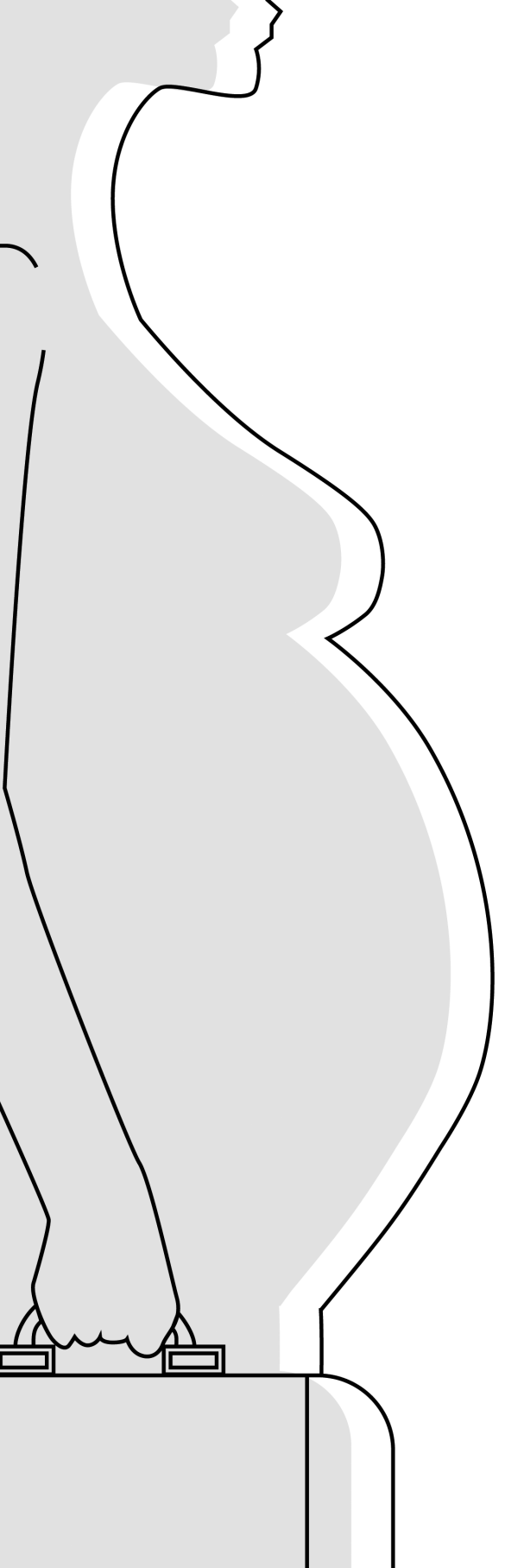
Abbreviations: FR, fecundability ratio; OR, Odds Ratio; fOR, fecundability odds ratio, CS, cross-sectional study; C, cohort study; CC, case-control study.

SUPPLEMENT 6. Results of the quality assessment of the 49 selected studies with information on associations between occupational exposure to various chemicals and time to pregnancy

Study (first author)	Total quality score	Research hypothesis	Study population	Selection bias	Exposure assessment	Outcome	Confounding	General opinion
Abell et al. 2000[32]	7	1	1	1	1	1	1	1
Ahlborg et al. 1996[70]	7	1	1	1	1	1	1	1
Apostoli et al. 2000[47]	7	1	1	1	1	1	1	1
Axmon et al. 2006[65]	6	1	1	0	1	1	1	1
Bonde et al. 1990[48]	6	1	1	1	1	0	1	1
Bretveld et al. 2008[36]	3	1	1	0	0	0	1	0
Bretveld et al. 2006[33]	4	1	1	0	0	0	1	1
Bretveld et al. 2008[44]	4	1	1	0	0	0	1	1
Bull et al. 1999[73]	6	1	1	1	0	1	1	1
Chen et al. 2002[72]	4	1	1	0	1	0	1	0
Cocco et al. 2005[74]	3	1	0	1	1	0	0	0
Curtis et al. 1999[42]	5	1	0	1	1	0	1	1
Dahl et al. 1999[63]	6	1	1	1	1	0	1	1
De Cock et al. 1994[37]	5	1	1	0	1	0	1	1
Eskenazi et al. 1991[58]	3	1	1	0	1	0	0	0
Figa-Talamanca et al. 2000[75]	7	1	1	1	1	1	1	1
Ford et al. 2002[77]	4	1	0	1	0	0	1	1
Fransman et al. 2007[71]	7	1	1	1	1	1	1	1
Greene et al. 2010[49]	5	1	1	1	0	1	0	1
Harley et al. 2008[43]	5	1	1	0	1	1	1	0
Hjollund et al. 1998[50]	6	1	1	0	1	1	1	1
Hooiveld et al. 2006[59]	5	1	1	0	1	0	1	1
Idrovo et al. 2005[34]	6	1	1	1	0	1	1	1
Joffe et al. 2003[51]	7	1	1	1	1	1	1	1
Kersemaekers et al. 1997[66]	4	1	1	1	0	0	1	0
Kolstad et al. 2000[60]	6	1	1	1	1	0	1	1
Larsen et al. 1998[38]	6	1	1	1	1	0	1	1
Lauria et al. 2006[35]	5	1	1	1	0	1	1	0
Luderer et al. 2004[61]	6	1	1	1	0	1	1	1
Modigh et al. 2002[76]	7	1	1	1	1	1	1	1
Peretz et al. 2009[67]	3	1	1	0	0	0	1	0
Petrelli et al. 2001[41]	5	1	0	1	1	0	1	1
Plenge-Bonig et al. 1999[78]	6	1	1	0	1	1	1	1

SUPPLEMENT 6 (continued). Results of the quality assessment of the 49 selected studies with information on associations between occupational exposure to various chemicals and time to pregnancy

Study (first author)	Total quality score	Research hypothesis	Study population	Selection bias	Exposure assessment	Outcome	Confounding	General opinion
Rachootin et al. 1983[80]	4	1	1	1	0	0	1	0
Rowland et al. 1994[64]	6	1	1	1	1	0	1	1
Sallmen et al. 1995[45]	5	1	0	1	1	0	1	1
Sallmen et al. 2003[39]	5	1	1	0	1	1	1	0
Sallmen et al. 1998[62]	6	1	1	1	1	0	1	1
Sallmen et al. 2000[52]	6	1	1	1	1	0	1	1
Sallmen et al. 1995[54]	6	1	1	1	1	0	1	1
Sallmen et al. 2008[55]	7	1	1	1	1	1	1	1
Schaumberg et al. 1989[69]	4	1	0	1	0	0	1	1
Shiau et al. 2004[53]	7	1	1	1	1	1	1	1
Spinelli et al. 1997[79]	4	1	1	1	0	0	1	1
Taskinen et al. 1999[56]	7	1	1	1	1	1	1	1
Thonneau et al. 1999[40]	6	1	1	1	1	1	1	0
Wennborg et al. 2001[57]	5	1	1	1	0	0	1	1
Wulff et al. 1999[46]	6	1	0	1	1	1	1	1
Zhu et al. 2005[68]	5	0	1	0	1	1	1	1



Chapter 2.2

Endocrine disruptors and time to pregnancy

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ABSTRACT

Objective: To study the influence of occupational exposure to endocrine disruptors (EDs) on time to pregnancy (TTP).

Design: Cross-sectional analysis within a prospective population-based cohort study.

Setting: Rotterdam, The Netherlands.

Patient(s): Mothers and fathers who filled out a questionnaire during mid-pregnancy (response 77% and 82% of enrolment, respectively) were selected if the pregnancy was planned and either parent performed paid employment. In total, 2774 mothers and 2728 partners were included in the statistical analyses.

Interventions(s): None.

Main outcome measure(s): Self-reported time to pregnancy (months).

Result(s): There was no correlation between maternal and paternal exposure, because kappa values for agreement for all exposure categories ranged from 0.03 to 0.13. Paternal occupational exposure to heavy metals (hazard ratio of pregnancy 0.83; 95% confidence interval 0.71-0.97), and overall exposure to EDs (hazard ratio 0.85; 95% confidence interval 0.75-0.96) was statistically significantly associated with an increased TTP. Maternal occupational exposure to all categories of EDs showed prolonged TTP, but the decreased hazard ratios were not statistically significant.

Conclusion: This birth cohort study provides indications for adverse effects of parental occupational exposure to EDs on TTP.

INTRODUCTION

Fertility problems are an important health issue: 10%-15% of couples have difficulties conceiving, or conceiving the number of children they want, and seek specialist fertility care at least once during their reproductive lifetime.[1] Time to pregnancy (TTP) was reported by Baird et al. as a good measure for estimating fecundability[2,3] and has recently been described as a sensitive and feasible method for studying effects of occupational exposures, as well as for monitoring of fecundity.[4] Endocrine disruptors (EDs), a group of substances that have the potential to alter the normal functioning of the endocrine system, are of growing concern.[5] It has been suggested that exposure to EDs in early pregnancy influences male reproductive development, leading to disorders such as low sperm count, subfertility, and testicular cancer.[6] Potential routes for exposure are food products, the environment, consumer products, and occupation.

The number of studies relating occupational ED exposure to TTP is limited. Several studies investigated distinct exposures in specific occupations in relation to TTP, most notably pesticide exposure among greenhouse and agricultural workers. Reviews by Roeleveld et al., Hanke et al., and Bretveld et al. found limited evidence for an influence of exposure to pesticides among fathers or mothers on reproduction.[7-9] Indications exist for delayed TTP after exposure to heavy metals or organic solvents, but the focus on either male or female exposure and subsequent lack of adjustment for partner's exposure makes the results difficult to interpret.[10]

The aim of the present study was to study the separate influence of maternal and paternal occupational exposure to a comprehensive set of potential EDs on TTP.

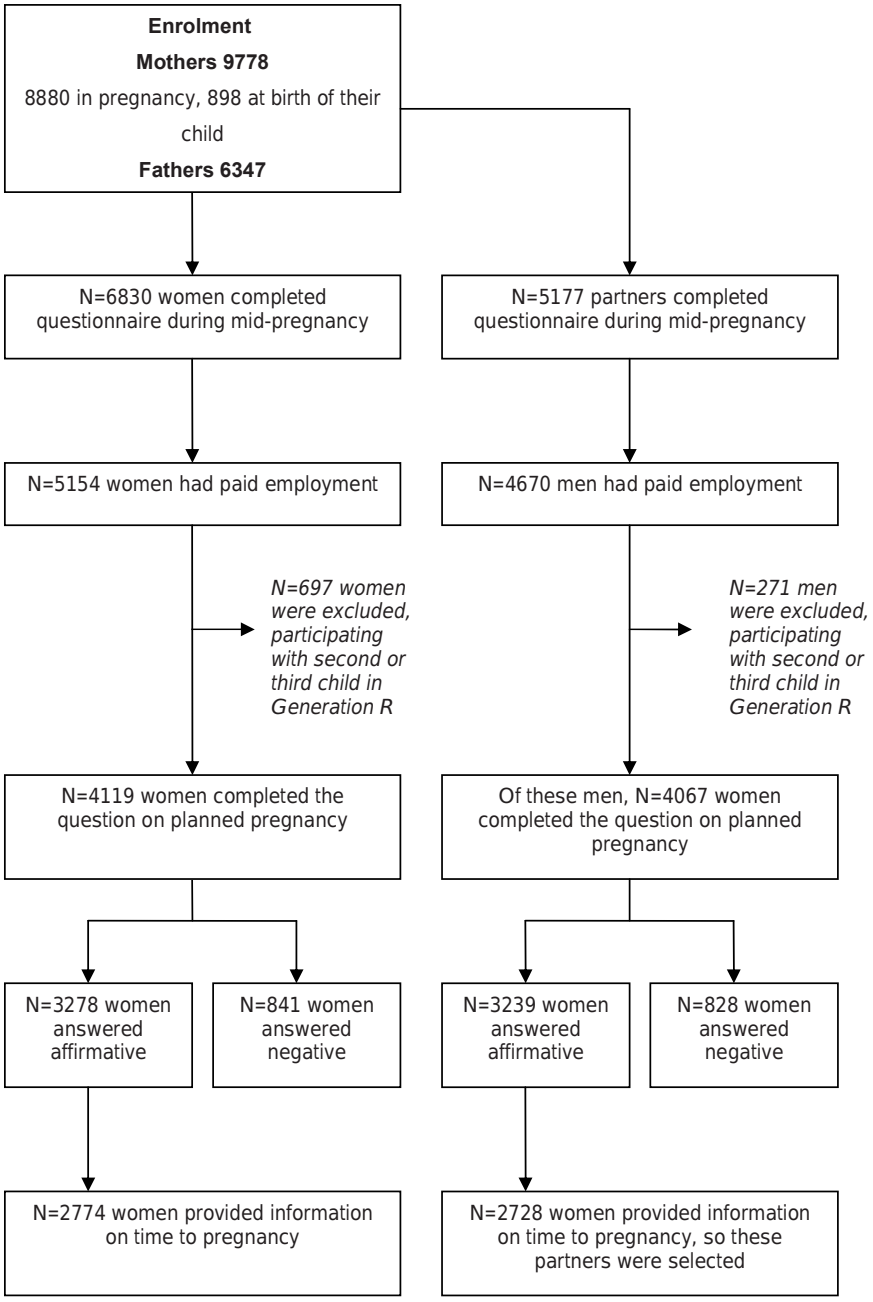
METHODS

Design and study population

The Generation R Study is a population-based prospective cohort study on growth, development, and health from early foetal life until young adulthood in Rotterdam, the Netherlands. The study design has been described in detail previously.[11,12] Briefly, all pregnant women who had an expected delivery date between April 2002 and January 2006 and living in the study area of Rotterdam were invited to participate. In total, 9778 pregnant women (response 61%; 8880 women during pregnancy and another 898 at birth of their child) and 6347 partners (response 71%) were enrolled. The study was approved by the Medical Ethics Committee at Erasmus University Medical Centre Rotterdam, The Netherlands. The information required for this study was collected in the questionnaire completed during mid-pregnancy by 6830 women (77% of enrolment) and 5177 partners (82% of enrolment). The questions on occupational status (paid employment), planned pregnancy (affirmative), and TTP were used for the selection of the study population. For each couple, we included the first pregnancy within the Generation R cohort in our study, because some women participated with more than one child in the study.

In total, 2774 women and 2728 partners were included in the analysis, the flowchart is depicted in Figure 1.

FIGURE 1. Flowchart of the selected study population



Time to pregnancy

The primary outcome in this study was TTP as a measure of fecundability. The questionnaire participants filled out during mid-pregnancy or thereafter at delayed enrolment included a question on the natural origin of the pregnancy (yes/no) and, in case of a positive answer, women with a planned pregnancy were asked about the number of months it took the couple to conceive.[13]

Occupation and working conditions

The questionnaire contained questions about work status, occupation, and working conditions and focussed on the start of the period of unprotected intercourse. Work status, based on a single question on the current economic status with seven categories (paid labour, self-employed, unemployed, disabled, homemaker, student, or other), was used to select women and men with paid employment. Questions on starting date of current work, quitting date, job title, type of business, name of employer, and activities in the job were used to classify jobs into the Dutch Classification of Occupations[14] and subsequently link these codes to standard occupational classification (SOC) codes and the job-exposure-matrix (JEM). The JEM is based on the judgement of experts who estimated the probability of exposure to ten categories of potential EDs, namely polycyclic aromatic hydrocarbons, polychlorinated organic compounds, pesticides, phthalates, organic solvents, bisphenol A, alkylphenolic compounds, flame retardants, metals, and miscellaneous agents,[15] for 353 job titles in three levels: 'unlikely'(0), 'possible'(1), and 'probable'(2). For this study we collate the last two categories into one category indicating the occurrence of exposure to EDs.

Potential confounding factors

Information on age, height, weight, education, country of origin, parity, smoking habits, and alcohol use was collected from the first questionnaire available. Maternal and paternal age was determined at intake in the study, and we calculated maternal and paternal age at start of the time to pregnancy period by subtracting the gestational age (in weeks) at intake and the TTP (in months). Educational level was defined as the highest educational program successfully completed and was categorised as low (primary school, lower vocational training, intermediate general school, three years general secondary school), mid-low (>three years general secondary school, intermediate vocational training), mid-high (higher vocational training, Bachelor's degree) or high (higher academic education). The country or origin of the pregnant mother was based on the country of birth of her parents, as defined by Statistics Netherlands. The country of origin assigned to non-natives (mother born abroad or at least one of the parents of the mother born abroad) is the country of the mother when both parents are born abroad in different countries, or when one parent is born abroad, the country of birth of that parent.[16] Three groups were defined: (1) Antilleans and Surinamese, (2) Turks and Moroccans, and (3) all other non-native groups. Smoking and alcohol use was assessed during early-pregnancy by a

dichotomous question asking whether the subject had smoked or drunk alcohol in the past two months.[17] The Body Mass Index (BMI) was calculated by weight divided by squared height, information available in the first questionnaire concerning the pre-pregnancy period.

Statistical analysis

The statistical analyses were performed by means of SAS version 9.2 (SAS Institute, Cary, NC) using a discrete proportional hazard model with TTP as time variable, including both natural and non-natural pregnancies. Censoring took place in case of a non-natural pregnancy at 12 months of TTP. Log-minus-log plots were made for the exposure category any EDs for mothers and fathers separately to inspect possible deviations from the proportional hazard assumption. The resulting hazard (HR) from the discrete proportional hazards model represents the fecundability of exposed subjects in a certain category of the JEM relative to non-exposed subjects in that category of the JEM. Initially, crude HRs with 95% confidence intervals were calculated for all non-occupational and occupational variables; second, multivariable analyses were performed. We selected all reported potential confounders, such as parental age, ethnicity, educational level, parity, BMI, alcohol and smoking. Variables were retained in the multivariable model as confounder when they changed the HR of ED exposure by more than 10%. Parental age as important confounder in many studies was included by default. The agreement between maternal and paternal occupational exposures, and between the various exposure categories was calculated by the weighted Cohen's Kappa.[18]

We carried out several sensitivity analyses. The first analysis evaluated whether women and men who started working in their current job during the TTP period differed from women and men starting before the TTP period. The second analysis investigated potential bias introduced owing to different exposure patterns among couples with complete TTP information and those with missing values on TTP as well as couples with a planned pregnancy and those with an unplanned pregnancy.[19]

RESULTS

The characteristics of the study population are shown in Table 1. For both mothers and fathers a lower education and overweight were associated with prolonged TTP (Table 2). The crude and adjusted HRs for exposure to EDs are shown in Table 3. The curves for exposed and non-exposed in the log-minus-log plots ran parallel, indicating that the proportional hazard assumption was met. Models were solely adjusted for parental age, since the other covariates did not change the HRs by more than 10%. Maternal occupational exposure to pesticides, phthalates, organic solvents, and alkylphenolic compounds showed a prolonged TTP. However, these associations failed to reach the conventional level of statistical significance of 0.05. Paternal occupational

TABLE 1. General characteristics of mothers and fathers enrolled in a prospective prenatally recruited birth cohort, Generation R

Individual characteristics		**Mothers** (n=2774)	**Fathers** (n=2728)
Age start TTP period (mean, SD)	years	30.57 (4.13)	32.67 (5.04)
Age start TTP period	< 25 years	276 (32.3%)	154 (5.6%)
	25-30 years	896 (32.3%)	619 (22.7%)
	30-35 years	1253 (45.2%)	1191 (43.7%)
	>35 years	349 (12.6%)	763 (28.0%)
Educational level	Low	324 (11.7%)	514 (18.8%)
	Mid-low	776 (28.0%)	672 (24.6%)
	Mid-high	704 (25.4%)	548 (20.1%)
	High	935 (33.7%)	934 (34.2%)
Ethnicity	Netherlands	1910 (68.9%)	1968 (72.1%)
	Surinam and Dutch Antilles	160 (5.8%)	168 (6.2%)
	Morocco and Turkey	194 (7.0%)	185 (6.8%)
	Other	494 (17.8%)	406 (14.9%)
Parity	First child	1872 (67.5%)	1831 (67.1%)
	Second child or higher	900 (32.4%)	895 (32.8%)
Body Mass Index at start TTP period	<25 kg/m^2	1797 (64.8%)	1365 (50.0%)
	25-30 kg/m^2	694 (25.0%)	1144 (41.9%)
	30-35 kg/m^2	197 (7.1%)	184 (6.7%)
	>35 kg/m^2	70 (2.5%)	32 (1.2%)
Smoking before pregnancy	yes	1079 (38.9%)	1100 (40.3%)
Alcohol use before pregnancy	yes	2137 (77.0%)	2379 (87.2%)
Time to pregnancy (TTP) (median, min-max)		3.0 (1.0-120.0)	3.0 (1.0-120.0)
Time to pregnancy	0-6 months	2102 (75.8%)	2083 (76.4%)
	6-12 months	457 (16.5%)	445 (16.3%)
	12-24 months	157 (5.7%)	144 (5.3%)
	24+ months	58 (2.1%)	56 (2.1%)

Values are absolute numbers (percentages) unless otherwise indicated.

exposure to heavy metals and any EDs was associated with prolonged TTP (HR 0.83; 95%CI 0.71-0.97 and HR 0.85; 95%CI 0.75-0.96, respectively).

The sensitivity analyses on start of work before or during the TTP period showed very similar HRs. The sensitivity analysis on potential bias due to missing TTP information or unplanned pregnancy showed that the likelihood of exposure to EDs was not associated with availability of TTP information and did not differ between couples with a planned pregnancy versus unplanned pregnancy or spontaneous vs. nonspontaneous conceived pregnancies. The possibility of a pregnancy planning bias was further investigated by a repeated analyses without

TABLE 2. Univariable analyses on fecundability ratios for non-occupational variables for mothers and fathers within the Generation R Cohort [a]

Individual characteristics		**Mothers** (n=2774)	**Fathers** (n=2728)
		HR 95% CI	HR 95% CI
Age at intake	<25 years	1.00	1.00
	25-30 years	1.13 (0.98-1.29)	1.10 (0.92-1.32)
	30-35 years	1.12 (0.98-1.28)	1.17 (0.99-1.39)
	>35 years	0.98 (0.83-1.15)	1.10 (0.92-1.32)
Educational level	Low	0.81 (0.71-0.92)*	0.86 (0.77-0.96)*
	Mid-low	0.89 (0.81-0.98)*	0.89 (0.80-0.98)*
	Mid-high	0.99 (0.89-1.09)	0.93 (0.84-1.04)
	High	1.00	1.00
Country of origin	Netherlands	1.00	1.00
	Surinam and Dutch Antilles	0.86 (0.73-1.01)	0.90 (0.77-1.06)
	Morocco and Turkey	1.00 (0.86-1.16)	1.06 (0.91-1.23)
	Other	0.94 (0.85-1.04)	0.95 (0.85-1.05)
Parity	First child	1.00	1.00
	Second child and higher	1.12 (1.03-1.21)*	1.10 (1.02-1.20)*
BMI	<25 kg/m^2	1.00	1.00
	25-30 kg/m^2	0.90 (0.82-0.98)*	0.93 (0.86-1.01)
	30-35 kg/m^2	0.88 (0.75-1.02)	0.83 (0.71-0.97)*
	>35 kg/m^2	0.82 (0.64-1.04)	0.73 (0.50-1.06)
Smoking	No	1.00	1.00
	Yes	0.96 (0.88-1.03)	0.98 (0.91-1.06)
Alcohol	No	1.00	1.00
	Yes	1.11 (1.00-1.22)*	1.03 (0.92-1.16)

[a] Data analysed using a Cox Proportional Hazards model (SPSS v17.0).

* p-value < 0.05.

TTP values of 0 and 1, which excluded 599 couples. This analysis showed in general similar HRs values, with changes of less than 10%.

For all groups of EDs, the agreement between maternal and paternal exposure was poor, with kappa values ranging between 0.03 and 0.13. When maternal and paternal occupational risk factors were mutually adjusted for each other within the same exposure category, the HRs remained largely the same. Maternal exposure to phthalates, solvents, and alkylphenolic compounds were interrelated (kappa values 0.47 to 0.77), and mutual adjustments by groups of exposure changed the HRs for specific groups by more than 10%, and the corresponding 95% CIs widened (data not shown).

TABLE 3. Univariable and multivariable analyses on fecundability ratios for various occupational exposure categories within the Job-Exposure-Matrix for mothers and fathers within the Generation R Cohort [a]

Exposure according to Job – Exposure – Matrix	Exposure prevalence	Mothers (n=2774)		Exposure prevalence	Fathers (n=2728)	
	(%)	Crude HR (95% CI)	Adjusted HR[b] (95%CI)	(%)	Crude HR (95%CI)	Adjusted HR[c] (95%CI)
Job exposure matrix						
PAH	0.9	0.87 (0.57-1.35)	0.88 (0.57-1.35)	4.7	0.85 (0.70-1.03)	0.84 (0.69-1.03)
Polychlorinated compounds	0.0	-	-	0.3	1.50 (0.69-3.26)	1.50 (0.69-3.25)
Pesticides	0.4	0.60 (0.32-1.14)	0.61 (0.32-1.15)	2.1	0.91 (0.68-1.21)	0.91 (0.68-1.21)
Phthalates	1.4	0.73 (0.52-1.03)	0.74 (0.52-1.04)	3.7	1.01 (0.81-1.26)	1.01 (0.81-1.26)
Organic solvents	4.5	0.86 (0.71-1.05)	0.86 (0.71-1.05)	7.2	0.89 (0.76-1.04)	0.89 (0.76-1.04)
Alkylphenolic compounds	2.9	0.81 (0.63-1.03)	0.81 (0.63-1.04)	1.9	0.83 (0.62-1.12)	0.83 (0.62-1.12)
Flame retardants	0.0	-	-	0.2	1.51 (0.47-4.85)	1.51 (0.47-4.83)
Metals	1.0	0.91 (0.60-1.36)	0.91 (0.61-1.37)	7.7	0.84 (0.72-0.98)*	0.83 (0.71-0.97)*
Any EDs	6.2	0.91 (0.76-1.08)	0.91 (0.77-1.08)	15.3	0.85 (0.76-0.96)*	0.85 (0.75-0.96)*

[a] Data analysed using a discrete proportional hazards model. (SAS v9.2).

[b] adjusted for maternal age at start of time to pregnancy period.

[c] adjusted for paternal age at start of time to pregnancy period.

* p value < 0.05.

DISCUSSION

In this population-based study, we found associations between paternal occupational exposure to heavy metals, and any EDs with a prolonged TTP. Maternal occupational exposure to EDs was not statistically significantly associated with a prolonged TTP.

Exposure assessment in this study was based on questions regarding several occupational characteristics in a 'general' questionnaire, which reduced the possibility of recall bias because the study subjects were not aware of the hypothesis tested. A recently updated JEM was used for exposure assessment; this approach assured that exposure was classified independently from the outcome, and blinded to participants - both aspects that avoid information bias. However, no specific data on occupational hygiene measurements from the companies or biomonitoring of the workers were available. Another shortcoming is that the JEM does not account for variability in tasks and working environment within job titles. Thus, the outcome of this matrix must be interpreted as exposure probabilities, which are a crude measure of exposure. Therefore, it is necessary to be cautious in interpreting the reported risk estimates.

Agreement between the different exposures was considerable for mothers for the categories phthalates, organic solvents, and alkylphenolic compounds, indicating that women exposed to one of these substances are likely to be exposed to the other substances as well. When we adjusted by group of exposure we noticed that the HRs changed by more than 10%. Because of these interrelationships among exposure groups, we could not disentangle the specific role of these groups of EDs in the observed prolonged TTP. Among fathers there was little overlap among the exposure categories. Furthermore, the analyses showed that no overlap existed between mothers and fathers exposed to EDs and, in addition, that mutual adjustments did not change the observed associations. This may suggest that in findings from previous research on occupational exposure of either females or males, the risk of residual confounding due to work of the partner can be largely ruled out.

Our main finding, that paternal occupational exposure to heavy metals and any EDs prolonged TTP, is partly in line with the current literature. For exposure to heavy metals, the comparison with the existing literature is complex, because we have studied heavy metals as a group, whereas in most other studies the effects of separate heavy metals are reported. For lead exposure, Sallmen et al. concluded that studies have consistently shown that lead exposure reduces fertility.[20] In a study by Bonde et al.,[21] a prolonged TTP was found among welders, but after adjustments for potential confounders this association was no longer statistically significant.

Study design issues play a role in interpreting the results from our study. A limitation of a population-based approach in studying occupational risk factors is that it may lack power to identify the role of a specific occupational exposure on fecundability, due to the low prevalence of exposure and the small to moderate effects of exposure on fecundability. On the other hand, population-based studies present estimates of the proportion of couples with fecundity

problems in the general population that may be attributed to particular occupational exposures. This information may be used to guide the need for preconception counselling of parents to be. Furthermore, the population-based approach with recruitment during the prenatal period allows adjustments for a large number of potential confounders.

A limitation of this study is the initial participation of 61% and the response on the mid-pregnancy questionnaire of mothers and fathers of 77% and 82%, respectively. Selective participation occurred, because mothers from ethnic minorities and lower socioeconomic status were less represented in the study population.[11] This selection toward a more affluent and healthy study population may have influenced the prevalence of exposure to EDs at the workplace but did not bias the results because exposure status was assessed independently from TTP. Additionally, educational level was not a confounding factor in the analyses. Furthermore, we choose to collate the two exposure categories 'possible' and 'probable' exposure into one category reflecting the presence of exposure; the analyses showed that the HRs were comparable, only because of the low prevalence of exposure, the CIs in the analyses with separate exposure categories were much larger. Several sensitivity analyses were carried out to assess the influence of the selection criteria applied to our study population. The sensitivity analyses on the start of the current occupation showed that restricting the analysis to couples who had started working before the start of their TTP resulted in similar HRs. Furthermore, we found that the exposure to EDs was not associated with TTP information and planning of pregnancy; thus, the restrictions to couples with complete TTP information and planned pregnancies will not have influenced the presented results. The exclusion of couples with a TTP of 0 or 1 from the analysis showed no differences in HRs, indicating little evidence for the presence of wantedness bias.

In conclusion, we found associations among men occupationally exposed to heavy metals and overall exposure to EDs with a prolonged TTP. Maternal occupational exposure to all categories of EDs showed prolonged TTP, but the decreased HRs were not statistically significant. There was no overlap in exposure patterns between mothers and fathers. These results indicate that working with EDs may carry a reproductive hazard that warrants further studies to investigate the specific role of EDs in reproductive health.

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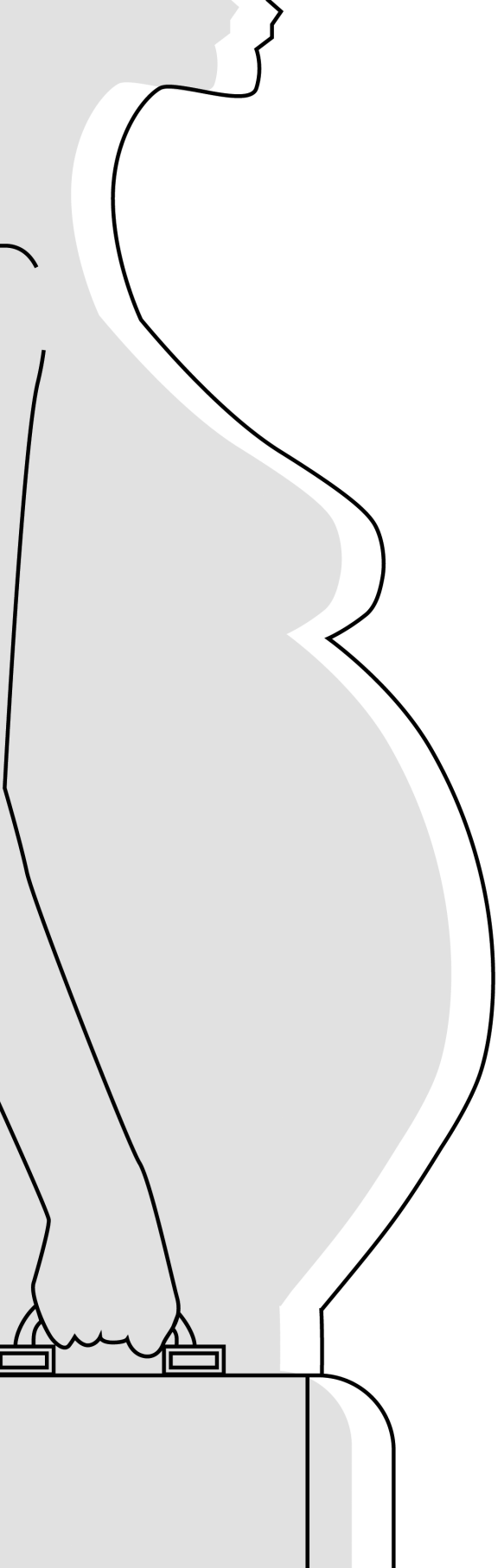

Chapter 2.3

Chemicals and foetal growth

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ABSTRACT

Background: Developmental diseases, such as birth defects, growth restriction and preterm delivery account for more than 25% of infant mortality and morbidity. Several studies have shown that exposure to chemicals during pregnancy is associated with adverse birth outcomes. The aim of this study was to identify whether occupational exposure to various chemicals might adversely influence intrauterine growth patterns and placental weight.

Methods: Associations between maternal occupational exposure to various chemicals and foetal growth were studied in 4680 pregnant women participating in a population-based prospective cohort study from early pregnancy onwards in the Netherlands (2002-2006), the Generation R Study. Mothers who filled out a questionnaire during mid-pregnancy (response 77% of enrolment), were included if they conducted paid employment during pregnancy and had a spontaneously conceived singleton liveborn pregnancy (n = 4680). A job-exposure-matrix was used, linking job titles to expert judgement on exposure to chemicals in the workplace. Foetal growth characteristics were repeatedly measured by ultrasound and were used in combination with measurements at birth. Placental weight was obtained from medical records and hospital registries. Linear regression models for repeated measurements were used to study the associations between maternal occupational exposure to chemicals and intrauterine growth.

Results: We observed that maternal occupational exposure to polycyclic aromatic hydrocarbons (PAHs), phthalates, alkylphenolic compounds, and pesticides adversely influenced several domains of foetal growth, being foetal weight, foetal head circumference, and foetal length. We found a significant association between pesticide and phthalate exposure with a decreased placental weight.

Conclusions: Our results suggest that maternal occupational exposure to several chemicals is associated with impaired foetal growth during pregnancy and a decreased placental weight. Further studies are needed to confirm these findings and to assess postnatal consequences.

INTRODUCTION

Developmental diseases, such as structural alterations (birth defects), functional alterations, growth restriction and preterm delivery, account for more than 25% of infant mortality and morbidity.[1,2] Foetal growth is generally assessed by surrogate measures, including length of gestation and foetal size, and these endpoints are important determinants of later health and morbidity.[3-5] Common risk factors for adverse foetal development include ethnicity,[6] smoking and alcohol use,[7] previous children with low birth weight or preterm birth, older maternal age, and low socioeconomic status.[8] Recently, it has been suggested that environmental risk factors and parental occupation may also play an important role.[9-11]

Women constitute a substantial part of the labour force in the European Union (EU). In 2010, about 58% of the women aged between 15-64 years had paid employment, which was a substantial increase from 54% in 2002.[12] With the increasing labour force participation among women in European countries, the likelihood that women will be exposed to a variety of chemical, physical, and psychological risk factors at work during pregnancy will also increase.[13] Although women in paid employment have better pregnancy outcomes than those without paid jobs,[14-16] certain work-related factors, such as exposure to chemicals,[17] physically demanding work[18] and psychological job strain[19] may adversely influence pregnancy outcome.

Exposure to chemicals during foetal development may increase the risk of adverse health consequences, including adverse birth outcomes, childhood morbidity, and adult disease and mortality.[2,20] Chemicals that have been associated with adverse foetal development are lead, and other heavy metals,[21,22] phthalates[23] and pesticides.[24-26] Chemicals can cross the placenta and enter the foetus, and a number of chemicals measured in maternal urine and serum have also been found in amniotic fluid, cord blood, and meconium.[27] A recent study by Woodruff et al. showed that pregnant women in the US were exposed to multiple chemicals.[28] The mechanism by which chemicals affect reproductive events are not completely understood, direct toxic effects may occur when normal processes such as differentiation, mitosis, meiosis, intracellular communication, DNA repair are altered. In this regard, the foetus is particularly vulnerable due to its fast growth, the process of cellular differentiation, the immaturity of their metabolic pathways, and the stage of development of vital organs.[29]

Since several studies have shown that exposure to chemicals during pregnancy adversely influence foetal development, as demonstrated by an increased occurrence of low birth weight, small-for-gestational-age and preterm delivery,[2,11,30] we expect that exposure to chemicals might already influence foetal growth in the different trimesters during pregnancy. Although birth outcomes are important from an obstetric perspective, they are rather crude measures of foetal growth during pregnancy.

The aim of this study was to identify, within a population-based prospective birth cohort study, whether occupational exposure to various chemicals might adversely influence intrauterine growth patterns and placental weight.

MATERIALS AND METHODS

Study design

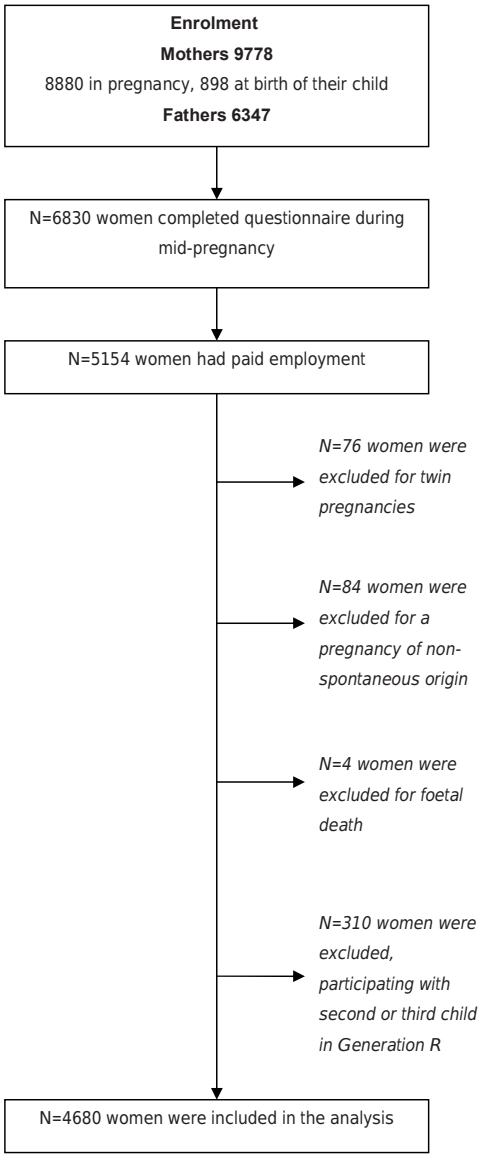
The Generation R Study is a population-based prospective cohort study on growth, development, and health from early foetal life until young adulthood in Rotterdam, the Netherlands. The study design has previously been described in detail.[31,32] Briefly, all pregnant women who had an expected delivery date between April 2002 and January 2006 and lived in the study area of Rotterdam were invited to participate. In total, 9778 pregnant women (response 61%) were enrolled in the study of which 8880 women were enrolled during pregnancy and another 898 at birth of their child. Extensive assessments were carried out during the first trimester (gestational age < 18 weeks), second trimester (gestational age 18-25 weeks), and third trimester (gestational age > 25 weeks), including physical examinations, questionnaires, interviews, and biological samples. The study was approved by the Medical Ethics Committee at Erasmus University Medical Centre Rotterdam, The Netherlands (MEC 198.782/2001/31).

The occupational information required for this study was collected in the questionnaire completed during mid-pregnancy, which was filled out by 6830 women (77% of enrolment). For this study we selected women who were prenatally enrolled, with paid employment before or during pregnancy, and with a spontaneously conceived singleton liveborn pregnancy. For each couple, we included the first pregnancy within the Generation R cohort in our study, since some women participated with more than one child in the study. Finally, the study population consisted of 4680 women, the flowchart of the study population is depicted in Figure 1. Our results are based on the second and third trimester ultrasonography measures in combination with birth outcomes.

Foetal ultrasounds

For this study we used the ultrasound measures of foetal head circumference, femur length, and estimated foetal weight, since these three measures are essential characteristics to describe foetal growth. Foetal ultrasound examinations were carried out in two dedicated research centres in each trimester of pregnancy. We measured foetal head circumference (HC), abdominal circumference (AC), and femur length (FL) to the nearest millimetre using standardised ultrasound procedures in the second (median 20.5, minimum-maximum 18.0-25.0 weeks) and third (median 30.4, minimum-maximum 25.8-37.0 weeks) trimester. Since use of the last menstrual period for pregnancy dating has several limitations,[33] and a large number of women in our study population did not know the exact date of their last menstrual period (76%), we used crown-rump length for pregnancy dating until a gestational age of 12 weeks (2308 women) and biparietal diameter for pregnancy dating thereafter (2372 women) in all women.[34,35] First trimester measurements (3459 women) were primarily used to establish gestational age and therefore not included in the growth analysis. Estimated foetal weight (EFW) was calculated using the formula by Hadlock et al.[36] Ultrasound examinations were performed using an Aloka

FIGURE 1. Flowchart of the study population



model SSD-1700 (Tokyo, Japan) or the ATL-Philips Model HDI 5000 (Seattle, WA, USA). Customised growth curves for the entire study population were constructed, and standard deviation (SD) scores for each individual women were calculated as deviation from the 'overall' average at that gestational week, and represent the equivalent z-scores.[33] The intraclass correlation coefficient of foetal growth measurements was 0.95, tested on 21 subjects, indicating a high reproducibility of foetal biometry measurements.[37]

Placenta and birth outcomes

Placental weight was obtained from medical records and hospital registries. Information about gender at birth, gestational age, weight, length, and head circumference at birth was obtained from medical records and hospital registries. For the analysis, we used birth weight, head circumference at birth, and length of the infant at birth.

Occupation and working conditions

The mid-pregnancy questionnaire contained questions about work status, occupation, and working conditions and focussed on the periconception and pregnancy period. Work status, based on a single question on the current economic status with seven categories: paid labour, self-employed, unemployed, disabled, homemaker, student, or other, was used to select women with paid employment. This question was followed by questions whether the mother had worked before conception in this current occupation, and the starting and (optional) stop date of this current occupation. We selected women who started working before conception and women who started working somewhere during the first trimester of pregnancy. Further questions on job title, type of business, name of employer, and activities in the job were used to classify jobs into the Dutch Classification of Occupations[38] and subsequently link these codes to a Job-Exposure-Matrix (JEM) for chemical exposure.[39] This new JEM was developed according to a general strategy, comprising of a literature search to identify chemicals, information gathering on occupations at risk, and literature on occupational settings in which the selected chemicals were encountered and exposure measurements were performed. This reference material served as a starting point for the expert assessment. Three experts were asked to estimate exposures based on their knowledge of tasks and working environment in various occupations. Finally, exposure probability scores were added based on the judgement of three experts. For various chemicals, subjects experience a certain level of exposure through diet, environment or widely used consumer products. The JEM exposure score refers to the probability of occupational exposure, which is assumed to exceed the background level in the general population. The exposure probability scores were assigned by means of consensus discussions in which the original scores were taken into account where possible, but no prior individual assessments were performed. The JEM comprises ten categories of chemicals, namely polycyclic aromatic hydrocarbons, polychlorinated organic compounds, pesticides, phthalates, organic solvents, bisphenol A, alkylphenolic compounds, flame retardants, metals, and miscellaneous agents.[39] For 353 job titles probability scores were classified in three levels: 'unlikely' (0), 'possible' (1), and 'probable' (2). Different country specific JEMs have been used in several studies and the JEM is a valuable tool for exposure assessment in epidemiological studies on the health risks of chemical exposure.[14,40-42] For this study we collated the last two categories into one category indicating the occurrence of exposure to chemicals.

Potential confounders

Information about maternal age, pre-pregnancy weight, educational level, ethnicity, parity, and folic acid supplement use was obtained by questionnaire at enrolment in the study. Maternal smoking habits and alcohol use were assessed on the basis of three questionnaires (in early, mid- and late pregnancy) and classified as no, until pregnancy was known, or during pregnancy.[43] Maternal height was measured at intake in the study. The questions on physical work load were obtained from the Dutch Musculoskeletal Questionnaire and concerned questions on long periods of standing, manually handling loads of 5 kg or more, manually handling loads of 25 kg or more, and night shifts. The presence of doctor-diagnosed preeclampsia, pregnancy induced hypertension, and diabetes gravidarum was retrieved from medical records and was based on the criteria of the International Society for the Study of Hypertension in Pregnancy.[44,45]

Statistical analysis

We assessed the associations between maternal occupational exposure to various chemicals and longitudinally measured SD scores of head circumference, length (second- and third trimester femur length and birth length), and weight (second- and third trimester estimated foetal weight and birth weight) using a mixed model for repeated measurements with an unstructured error term. This is a commonly used method to analyse data from longitudinal studies.[46] First, customised growth curves for the entire study population were constructed, and standard deviation (SD) scores for each individual women were calculated as deviation from the 'overall' average at that gestational week.[33] This approach resembles the common measure weight-for-age z-scores (WAZ), used in international studies on undernutrition and child mortality in order to increase comparability of effects independent of underlying differences in distributions.[47] These gestational age adjusted SD scores were used as parameters of foetal growth, the dependent variables in the statistical analyses. Second, a linear model was used to study the influence of occupational exposure to chemicals on these gestational age adjusted SD scores. The final model can be written as (for example for foetal weight): SD score of foetal weight = $ß_0 + ß_1$ x gawks + $ß_2$ x exposuregroup + $ß_3$ x gawks x exposuregroup (gawks=gestational age in weeks). In this model, $ß_0$ reflects the intercept and $ß_2$ expresses the systematic difference between exposed en non-exposed groups. The coefficient $ß_3$ reflects whether exposed and non-exposed foetus grow at the same rate over time. The later coefficient is the main interest of this analysis, since it represents the average decline or increase in SD for foetal weight per gestational week for exposed women versus non-exposed women. Different beta coefficients of interaction were estimated for weight, head circumference, and length, representing growth velocity for several domains of foetal growth. The regression models were adjusted for lifestyle and socioeconomic confounders used in previous studies on maternal occupational exposure[14,42] and known determinants of foetal growth: maternal age, educational level, ethnicity, parity, pre-pregnancy weight, height at intake, smoking during pregnancy, alcohol use during pregnancy, folic acid supplement use, foetal gender, physically

demanding work (long periods of standing, handling of loads > 5 kg, handling of loads > 25 kg, and night shifts), and pregnancy complications (preeclampsia, pregnancy induced hypertension, and diabetes gravidarum). For the important confounders ethnicity and educational level, potential interaction with exposure was investigated for each multivariable model with a significant effect of exposure on foetal growth.

Missing values in covariates were handled by multiple imputations (MCMC method) by generating five independent datasets for all analyses. Imputations were based on the relations between all covariates included in this study and the threshold for imputation was set on a maximum of 30% of missing values. We used the pooled adjusted effect estimates to generate the Figures 2-4. No differences were observed between analyses with imputed missing data or complete cases only. We performed a sensitivity analysis in order to evaluate whether women who started working in their current job before conception differed from women who started working during pregnancy. All levels of associations are presented with their 95% confidence intervals. The repeated measurement analyses were conducted with the Proc Mixed module of the Statistical Analysis System (version 9.2; SAS Institute Inc, Cary NC).

RESULTS

Table 1 shows the baseline characteristics of the study population. The mean age of the women at intake in the study was 31.1 years. Of all women, 30.3% had completed high education and the largest group was from Dutch origin (64.0%). The majority of women were nulliparous (63.9%). A total of 11.7% of the mothers continued smoking and 39.4% of the mothers continued drinking alcohol after the pregnancy was known. According to the JEM, 1.3% of the women were exposed to polycyclic aromatic hydrocarbons (PAHs), 0.5% to pesticides, 1.5% to phthalates, 4.7% to organic solvents, 3.3% to alkylphenolic compounds, 1.1% to metals, and 6.7% to any chemicals. In total, 4197 (89.7%) women visited our clinic for second trimester ultrasonography, and 4294 (91.8%) for third trimester ultrasonography. The median gestational age at birth was 40.1 weeks (minimum 22.7, maximum 43.4 weeks), while mean birth weight was 3450 grams (standard deviation 549 grams). Slightly more than 50% of the infants were boys. The three characteristics of foetal growth were interrelated with the highest association

TABLE 1. Baseline characteristics of pregnant women participating in a birth cohort study, The Generation R Study (n = 4680)

Variables	Results
Maternal characteristics	
Age at intake (yrs)	31.08 (4.56)
Weight before pregnancy (kg)	64.00 (34.00-145.00)
Height measured at intake (cm)	168.80 (7.12)

Educational level	Low	653 (14.0%)
	Mid-low	1333 (28.5%)
	Mid-high	1129 (24.1%)
	High	1419 (30.3%)
	Missing	146 (3.1%)
Ethnicity	Netherlands	2993 (64.0%)
	Surinam and Dutch Antilles	380 (8.1%)
	Marocco and Turkey	328 (7.0%)
	Other	885 (18.9%)
	Missing	94 (2.0%)
Parity	Nulliparous	2992 (63.9%)
	Multiparous	1565 (33.4%)
	Missing	123 (2.6%)
Smoking	Yes, during pregnancy	546 (11.7%)
	Yes, until pregnancy was known	355 (7.6%)
	No	3031 (64.8%)
	Missing	748 (16.0%)
Alcohol	Yes, during pregnancy	1846 (39.4%)
	Yes, until pregnancy was known	587 (12.5%)
	No	1524 (32.6%)
	Missing	723 (15.4%)
Folic acid use	No	580 (12.4%)
	Yes, post conception start	1163 (24.9%)
	Yes, preconception start	1735 (37.1%)
	Missing	1202 (25.7%)
Occupational characteristics		
Exposure to:		
PAH		63 (1.3%)
Pesticides		23 (0.5%)
Phthalates		68 (1.5%)
Organic solvents		221 (4.7%)
Alkylphenolic compounds		156 (3.3%)
Metals		52 (1.1%)
Any chemicals		313 (6.7%)
Growth outcomes		
Second trimester ultrasonography		4197 (89.7%)
Third trimester ultrasonography		4294 (91.8%)
Birth outcomes		
Gestational age at birth (wk)		40.14 (22.71-43.43)
Birth weight (grams)		3449.81 (549.28)
Male		2365 (50.5%)
Head circumference at birth (mm)		33.89 (1.65)
Length at birth (mm)		50.33 (2.38)

Values are means (standard deviation) for normal distributed continuous variables or medians (minimum-maximum) for skewed distributed continuous variables, and absolute numbers (percentages) for categorical variables.

TABLE 2. Associations between occupational exposure to chemicals and placental weight among pregnant women participating in a birth cohort study

Occupational chemical exposure	Placental weight (grams)	
	Crude#	Adjusted†
Exposure to:		
PAH	-21.21 (-65.17; 22.75)	-7.64 (-52.03; 36.76)
Pesticides	-74.84 (-138.34; -11.35) *	-65.90 (-129.86; -1.94) *
Phthalates	-59.55 (-98.11; -21.00) *	-45.88 (-85.15; -6.60) *
Organic solvents	-17.74 (-39.21; 3.74)	-10.00 (-32.36; 12.36)
Alkylphenolic compounds	-15.81 (-41.01; 9.39)	-5.43 (-32.03; 21.16)
Metals	-37.14 (-80.53; 6.26)	-35.22 (-78.54; 8.09)
Any chemicals	-18.71 (-37.20; -0.22) *	-11.03 (-30.28; 8.23)

Results from simple and multiple linear regression analysis. Values are regression coefficients (95% confidence intervals) and reflect the difference in grams for placental weight between women exposed to chemicals in the workplace compared to non-exposed women. Based on 3185 measurements of placental weight.
adjusted for gestational age at birth
† adjusted for gestational age at birth, maternal age, educational level, ethnicity, foetal gender, weight before pregnancy, height at intake, smoking during pregnancy, alcohol use during pregnancy, folic acid use, parity, long periods of standing, handling loads of >5 kg, handling loads of >25kg, night shifts, preeclampsia, pregnancy induced hypertension, and diabetes gravidarum.
* P-value < 0.05.

between foetal weight and length (Pearson correlation coefficient r = 0.59, at birth) and the smallest association between head circumference and length (r = 0.43, at birth).

Table 2 shows the results of the linear regression analysis on occupational exposure to chemicals and placental weight. Women occupationally exposed to pesticides and phthalates showed a significantly lower placental weight compared with non-exposed women, respectively 65.90 grams for pesticides (95%CI -129.86;-1.94) and 45.88 grams for phthalates (95%CI -85.15;-6.60).

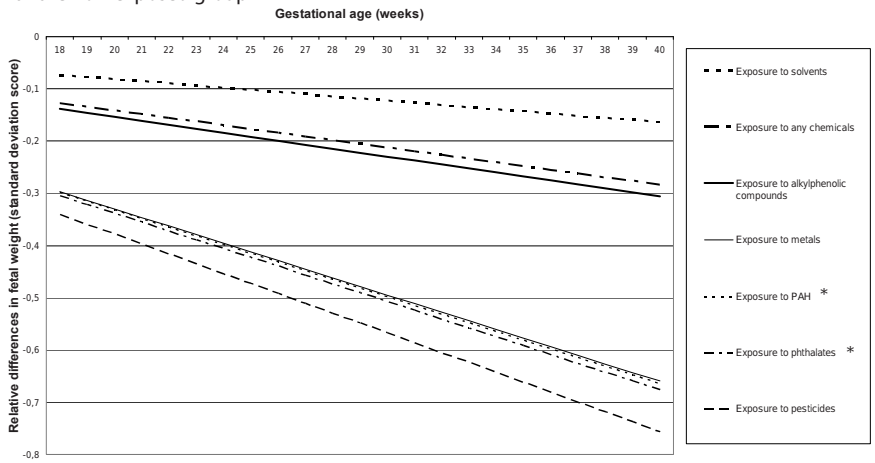
Table 3 shows the results of the univariable and multivariable longitudinal models for the associations between occupational exposure to various chemicals and foetal weight, head circumference, and foetal length. The average decline in standard deviation per gestational week is graphically illustrated in Figure 2-4. Maternal occupational exposure to several chemicals showed similar trends with lower growth rates for all three parameters. Women occupationally exposure to polycyclic aromatic hydrocarbons and phthalates showed significant lower foetal weight growth rates (average decline in SD per gestational week 0.01660 for PAHs and 0.01691 for phthalates) compared to non-exposed mothers, adjusted for potential confounders. In the fully adjusted model, the following covariates statistically significantly influenced foetal growth in order of decreasing importance: weight, height, parity, smoking, ethnicity, and diabetes gravidarum, but adjustments did not change the effect estimates of chemical exposure on foetal growth (Supplement 1). No interaction for exposure with ethnicity and educational level was observed in the multivariable models, indicating that ethnicity and education do not

TABLE 3. Association between occupational chemical exposure and foetal weight, foetal head circumference, and foetal length among pregnant women participating in a birth cohort study

Occupational exposure	Foetal weight			Foetal head circumference			Foetal length		
	Unadjusted estimate	Adjusted estimate	Standard error	Unadjusted estimate	Adjusted estimate	Standard error	Unadjusted estimate	Adjusted estimate	Standard error
Exposure to:									
PAH	-0.01647 *	-0.01660 *	0.00798	-0.01053	-0.01056	0.01114	-0.00328	-0.003139	0.01020
Pesticides	-0.01892	-0.01891	0.01274	-0.02619	-0.02603	0.01703	-0.03610 *	-0.035071 *	0.01605
Phthalates	-0.01675 *	-0.01691 *	0.00744	-0.01632	-0.01553	0.00982	-0.01845 *	-0.018183 *	0.00908
Organic solvents	-0.00411	-0.00410	0.00424	-0.00975	-0.00902	0.00560	-0.00743	-0.007048	0.00521
Alkylphenolic compounds	-0.00757	-0.00766	0.00500	-0.01834 *	-0.01752 *	0.00661	-0.00954	-0.008990	0.00621
Metals	-0.01682	-0.01649	0.00872	-0.00937	-0.00888	0.01163	-0.01246	-0.012172	0.01087
Any chemicals	-0.00712	-0.00710	0.00363	-0.00912	-0.00861	0.00482	-0.00520	-0.004850	0.00449

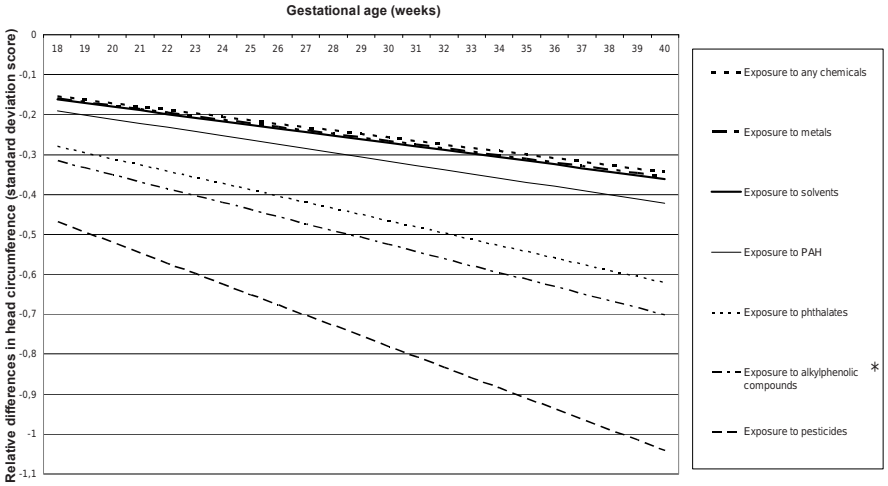
The beta coefficients represent the average decline in standard deviation per gestational week for foetal weight, head circumference and foetal length. Estimates are adjusted for the following confounders: maternal age, educational level, ethnicity, foetal gender, weight before pregnancy, height at intake, smoking during pregnancy, alcohol use during pregnancy, folic acid use, parity, long periods of standing, handling loads of >5 kg, handling loads of >25kg, night shifts, preeclampsia, pregnancy induced hypertension, and diabetes gravidarum.
* p-value > 0.05.

FIGURE 2. Adjusted relative differences in foetal weight (SD scores) in various chemical groups compared with the non-exposed group



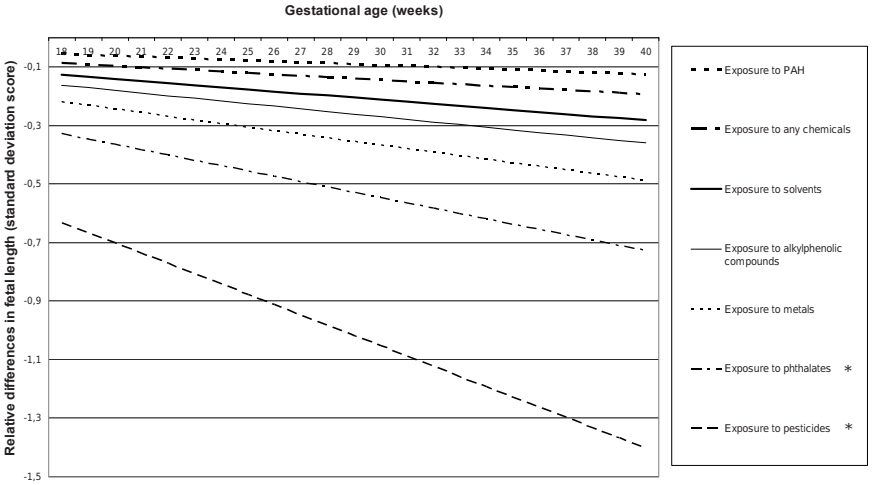
Values are based on repeated linear regression models and reflect the difference in SD score of foetal weight measurements (based on 12748 measurements) in the offspring of mothers occupationally exposed to various groups of chemicals compared to the offspring of non-exposed mothers. The reference value is a SD score of 0. * P-value < 0.05. Estimates are adjusted for the following confounders: maternal age, educational level, ethnicity, foetal gender, weight before pregnancy, height at intake, smoking during pregnancy, alcohol use during pregnancy, folic acid use, parity, long periods of standing, handling loads of >5 kg, handling loads of >25kg, night shifts, preeclampsia, pregnancy induced hypertension, and diabetes gravidarum.

FIGURE 3. Adjusted relative differences in head circumference (SD scores) in various chemical groups compared with the non-exposed group



Values are based on repeated linear regression models and reflect the difference in SD score of foetal head circumference measurements (based on 10789 measurements) in the offspring of mothers occupationally exposed to various groups of chemicals compared to the offspring of non-exposed mothers. The reference value is a SD score of 0. * P-value < 0.05. Estimates are adjusted for the following confounders: maternal age, educational level, ethnicity, foetal gender, weight before pregnancy, height at intake, smoking during pregnancy, alcohol use during pregnancy, folic acid use, parity, long periods of standing, handling loads of >5 kg, handling loads of >25kg, night shifts, preeclampsia, pregnancy induced hypertension, and diabetes gravidarum.

FIGURE 4. Adjusted relative differences in foetal length (SD scores) in various chemical groups compared with the non-exposed group



Values are based on repeated linear regression model and reflect the difference in SD score of foetal length measurements (based on 11401 measurements) in the offspring of mothers occupationally exposed to various groups of chemicals compared to the offspring of non-exposed mothers. The reference value is a SD score of 0. * P-value < 0.05. Estimates are adjusted for the following confounders: maternal age, educational level, ethnicity, foetal gender, weight before pregnancy, height at intake, smoking during pregnancy, alcohol use during pregnancy, folic acid use, parity, long periods of standing, handling loads of >5 kg, handling loads of >25kg, night shifts, preeclampsia, pregnancy induced hypertension, and diabetes gravidarum.

moderate or explain the observed associations between occupational exposure and foetal growth parameters.

For foetal head circumference, only maternal occupational exposure to alkylphenolic compounds showed a statistically significant lower growth rate (-0.0175 SD per gestational week) compared to non-exposed mothers, adjusted for potential confounders. For foetal length, we observed statistically significant lower growth rates between mothers occupationally exposed to pesticides and phthalates (-0.0361 SD per gestational week, and -0.0185 SD per gestational week, respectively) compared to non-exposed mothers, with a much steeper decline during the course of pregnancy for pesticides than for other occupational chemicals.

In total, 4177 (89.3%) women filled out the question concerning the starting date of their current occupation, 4068 women (97.4%) started working before conception, whereas 109 (2.6%) women started working somewhere during their first trimester of pregnancy. In the sensitivity analyses no differences in effect estimates were observed between women who started working before conception compared to women who started working during the first trimester of pregnancy. The differences in standard deviation scores for all foetal growth characteristics for unadjusted model, the adjusted model (pooled estimates), and for the five multiple imputation models are shown in Supplement 1. Supplement 2 and 3 show the individual data points of

exposed and non-exposed women for foetal weight and foetal head circumference in the second trimester, third trimester and at birth.

DISCUSSION

This large population-based prospective cohort study showed that maternal occupational exposure to several chemicals, such as polycyclic aromatic hydrocarbons (PAHs), phthalates, alkylphenolic compounds and pesticides during pregnancy, adversely influenced their foetal growth rates of weight, head circumference and length. These differences in foetal growth rates could already be demonstrated during pregnancy, and were partly reflected in a decreased placental weight. These findings suggest that early exposure during the critical window of foetal development is crucial.

In this study we used ultrasound measurements for pregnancy dating,[34,35] this method appears to be superior to dating based on the last menstrual period.[33] A disadvantage of pregnancy dating by ultrasound is that growth variations in crown-rump length and biparietal diameter in early pregnancy are assumed to be zero, impairing detailed analysis on foetal growth in the first trimester. In a sensitivity analyses on the subset of women with a certain last menstrual period and regular cycle (n = 1221), the direction of the effect estimates did not change. Reference curves for foetal growth were constructed for our cohort, which enables linear analyses of foetal growth characteristics. These curves are based on a large, urban, non-hospital based population, which makes these curves generalisable to normal foetal development in industrialised countries.[33] For the repeated measurements concerning foetal length, we used the SD score of birth length in combination with SD scores of femur length in second and third trimester in order to assess relative changes in foetal skeletal growth. However, the results should be interpreted with caution, since these measurements reflect different body parts. The repeated measurements based on gestational age adjusted SD scores were used in previous studies within the same cohort.[7,48] This method enables us to identify pathological smallness instead of constitutional smallness, which may be normal intrauterine growth. The advantage of SD scores as relative measure of difference is that the SD scores can be used in linear regression models, whereas absolute differences in foetal growth were highly skewed since growth curves during pregnancy have a typical parabolic shape that must be described by fractional polynomials instead of normal distributions. We demonstrated two of these curves with absolute differences in Supplement 2 and 3.

The strength of this study is the population-based approach with recruitment during the prenatal period and the availability of a large number of potential confounders. A limitation of this study is the selective participation with mothers from ethnic minorities and with lower socio-economic status less represented in the study population.[31] This selection may have influenced the prevalence of exposure to chemicals at the workplace, but bias is unlikely since

exposure status was assessed independently from and prior to the foetal growth characteristics by a recently updated job-exposure-matrix (JEM). This approach assured that exposure status was blinded to participants and researchers, both aspects which avoid information bias. The characterisation of exposure in the JEM must be interpreted as exposure probabilities, which are only a crude measure of exposure, which have to be interpreted with caution. Background exposure to various chemicals through diet and environment may occur. Previous research within the Generation R Study,[49] but also within the NHANES national survey showed that almost all pregnant women are exposed to chemicals, and that levels are comparable between pregnant and non-pregnant women.[28] However, there is reason to believe that occupational exposure is generally much higher than background exposure through diet and environment.[50] For example, for phthalates, Hines et al. showed that for several occupations the urinary phthalate concentrations exceeded the levels of the general population.[51] However, biomonitoring data comparing occupational exposures with exposure from non-occupational sources are scarce. In the current study we did not assess background exposure and, thus, it is not possible to distinguish the importance of different routes of exposure. Since it is unlikely that the widespread environmental exposure is associated with occupational exposure in specific jobs, background exposure will most likely not confound the observed relation between occupational chemical exposure and foetal growth.

Furthermore, the JEM does not contain specific chemicals, but only contains broad groups of chemicals, and the mechanisms of action can vary between specific chemicals in a group. A major drawback of JEMs is that they do not account for variability in tasks and working environments within job titles. However, from the task description, it may become clear that some subjects within a specific job title, for example subjects who have odd jobs around a farm (feeding animals) are less likely to be exposed to pesticides. The overlap between the categories phthalates, organic solvents, and alkylphenolic compounds was considerable for mothers (kappa values 0.47 to 0.77), indicating that women exposed to one of these substances were likely to be exposed to other substances as well. We must conclude that due to this interrelationship among exposure groups, it was not possible to disentangle the specific role of phthalates and alkylphenolic compounds in the observed lower foetal growth rates.

Women with lower education and women from ethnic minorities were more often exposed to chemicals in the workplace, but in our study this did not introduce confounding. As can be seen from Table 2 and Supplements, adjustments for education and ethnicity only slightly changed our effect estimates. Even though we were able to control for a large number of potential confounders, residual confounding cannot be ruled out completely. In this study we used multiple imputation for missing values in covariates. This reduces selection bias due to non-random missing in the covariates.

In this study we measured foetal growth, comprising three characteristics of foetal growth, namely weight, head circumference and length. Intrauterine growth restriction has been classified as symmetric and asymmetric, although the clinical relevance of this concept is

controversial.[52] Recent studies have shown that asymmetric foetal growth is associated with an increased neonatal morbidity.[53] Although it proved to be too difficult to distinguish between symmetric and asymmetric growth restriction in our study, we hypothesised that the comparable effects of occupational exposure to chemicals on all characteristics of foetal growth might be suggestive for symmetric growth restriction.

Several chemicals were associated with impaired foetal weight, resulting in a decrease in SD at birth varying between 0.2 and 0.7. This corresponds to approximately 100-400 grams difference in birth weight. The effect of occupational exposure to chemicals seems of similar magnitude than other well-known lifestyle factors, such as smoking, alcohol use, and caffeine intake. Bakker et al. showed a reduction of 0.3 SD in birth weight for mother who consumed caffeine > 6 units/day.[48] Jaddoe et al. showed that smoking impaired foetal growth, in particular head circumference, femur length, and abdominal circumference, with 0.1-0.3 SD.[7] However, the population attributable fraction is low, due to the low prevalence of exposure to these chemicals compared to other well-known lifestyle factors.

Workplace health is an important topic since women who intend to become pregnant and pregnant women are at risk for adverse pregnancy outcomes, thus, it is important to identify occupational related risk factors for prevention. Occupations in which women have a high exposure probability are agricultural and horticultural workers (pesticide exposure), hairdressers, beauticians, furniture makers (phthalate exposure), and cleaners (alkylphenolic compounds). Since the effects of occupational exposures on foetal growth are considerable, one could argue that pregnant women working in agriculture or horticultural trades must be informed about the risks of pesticide exposure in the workplace. However, the underlying mechanism of these exposures is largely unclear, and results from earlier studies are conflicting, which poses further research into this important topic.

This study supports existing evidence from human studies regarding occupational exposures and adverse pregnancy outcomes.[30] Although the chemicals in our study were considered to be potential endocrine disrupters, is remains to be established whether the mode of action is through endocrine disruption. A recent review by Caserta et al. summarises the literature regarding exposure to endocrine disrupting chemicals on pregnancy outcome.[54] They conclude that epidemiological studies on endocrine disruptors are not always consistent. This is further illustrated by occupational studies, for example in hairdressers, that show conflicting results.[55-57] Further studies are urgently needed to identify the molecular basis of the effects, to study the epigenetic effects of these exposures, and to develop strategies to prevent exposure to these agents to improve birth outcomes.[58]

Our results suggest that maternal occupational exposure to several chemicals adversely influence foetal growth patterns. Further studies are needed to confirm these findings and to identify potential targets for prevention.

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SUPPLEMENT 1. Overview of the estimates in univariable and multivariable models for SD scores for foetal weight, head circumference and length of exposed mothers versus non-exposed mothers

TABLE 1. Foetal weight

	Unadjusted estimate	p-value	Adjusted estimate	p-value
Exposure to:				
PAH	-0.01647	0.0390	-0.016598	0.0375
Pesticides	-0.01892	0.1375	-0.018908	0.1379
Phthalates	-0.01675	0.0244	-0.016907	0.0231
Organic solvents	-0.00411	0.3321	-0.004100	0.3334
Alkylphenolic compounds	-0.00757	0.1305	-0.007659	0.1258
Metals	-0.01682	0.0537	-0.016494	0.0585
Any chemicals	-0.00712	0.0495	-0.007095	0.0504

TABLE 2. Foetal head circumference

	Unadjusted estimate	p-value	Adjusted estimate	p-value
Exposure to:				
PAH	-0.01053	0.3451	-0.010555	0.3433
Pesticides	-0.02619	0.1242	-0.026027	0.1264
Phthalates	-0.01632	0.0972	-0.015530	0.1138
Organic solvents	-0.00975	0.0817	-0.009022	0.1068
Alkylphenolic compounds	-0.01834	0.0056	-0.017517	0.0080
Metals	-0.00937	0.4210	-0.008884	0.4449
Any chemicals	-0.00912	0.0588	-0.008605	0.0742

TABLE 3. Foetal length

	Unadjusted estimate	p-value	Adjusted estimate	p-value
Exposure to:				
PAH	-0.00328	0.7483	-0.003139	0.7583
Pesticides	-0.03610	0.0247	-0.035071	0.0289
Phthalates	-0.01845	0.0425	-0.018183	0.0452
Organic solvents	-0.00743	0.1544	-0.007048	0.1763
Alkylphenolic compounds	-0.00954	0.1251	-0.008990	0.1479
Metals	-0.01246	0.2526	-0.012172	0.2629
Any chemicals	-0.00520	0.2470	-0.004850	0.2796

Estimates are adjusted for the following confounders: maternal age, educational level, ethnicity, foetal gender, weight before pregnancy, height at intake, smoking during pregnancy, alcohol use during pregnancy, folic acid use, parity, long periods of standing, lifting >5 kg at work, lifting >25kg at work, night shifts, preeclampsia, pregnancy induced hypertension, and diabetes gravidarum.

Overview of the estimates of SD scores for foetal weight, head circumference and length of exposed mothers versus non-exposed mothers in the multiple imputation models.

TABLE 1. Foetal weight

	MI 1	MI 2	MI 3	MI 4	MI 5	Pooled adjusted estimate	p-value
Exposure to:							
PAH	-0.01658	-0.01658	-0.01657	-0.01661	-0.01664	-0.016598	0.0375
Pesticides	-0.01886	-0.01883	-0.01870	-0.01900	-0.01914	-0.018908	0.1379
Phthalates	-0.01688	-0.01694	-0.01684	-0.01692	-0.01696	-0.016907	0.0231
Organic solvents	-0.00409	-0.00410	-0.00410	-0.00412	-0.00410	-0.004100	0.3334
Alkylphenolic compounds	-0.00765	-0.00766	-0.00763	-0.00769	-0.00766	-0.007659	0.1258
Metals	-0.01652	-0.01644	-0.01641	-0.01655	-0.01655	-0.016494	0.0585
Any chemicals	-0.00709	-0.00709	-0.00709	-0.00712	-0.00709	-0.007095	0.0504

TABLE 2. Foetal head circumference

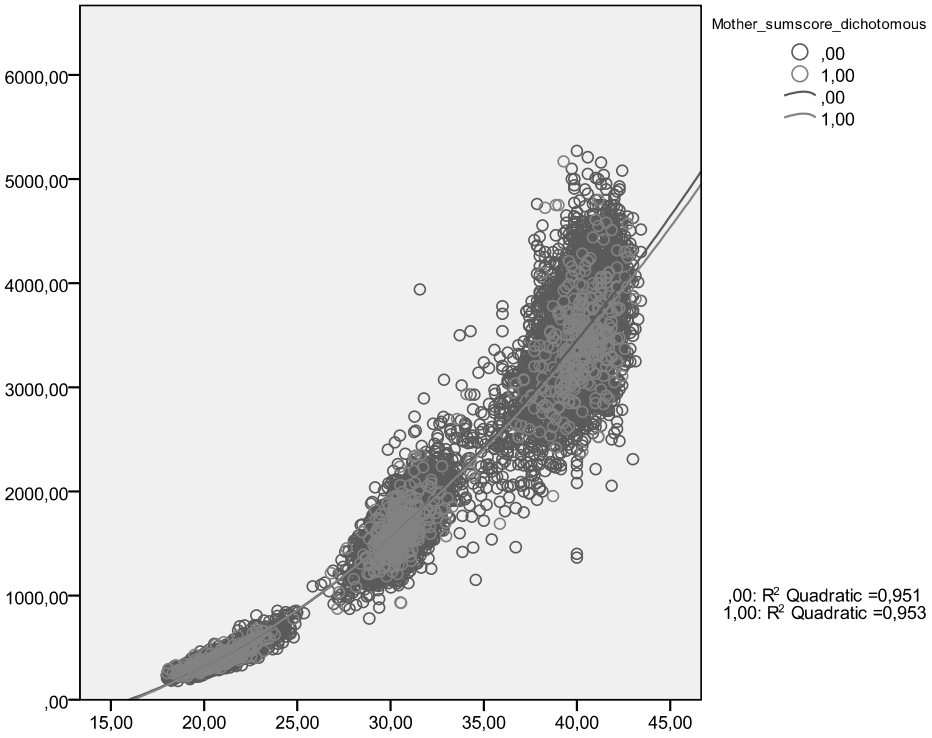
	MI 1	MI 2	MI 3	MI 4	MI 5	Pooled adjusted estimate	p-value
Exposure to:							
PAH	-0.01061	-0.01061	-0.01052	-001045	-0.01059	-0.010555	0.3433
Pesticides	-0.02572	-0.02647	-0.02577	-0.02578	-0.02638	-0.026027	0.1264
Phthalates	-0.01543	-0.01561	-0.01548	-0.01550	-0.01564	-0.015530	0.1138
Organic solvents	-0.00905	-0.00902	-0.00895	-0.00899	-0.00909	-0.009022	0.1068
Alkylphenolic compounds	-0.01754	-0.01755	-0.01743	-0.01748	-0.01760	-0.017517	0.0080
Metals	-0.00880	-0.00920	-0.00865	-0.00878	-0.00899	-0.008884	0.4449
Any chemicals	-0.00862	-0.00865	-0.00853	-0.00859	-0.00863	-0.008605	0.0742

TABLE 3. Foetal length

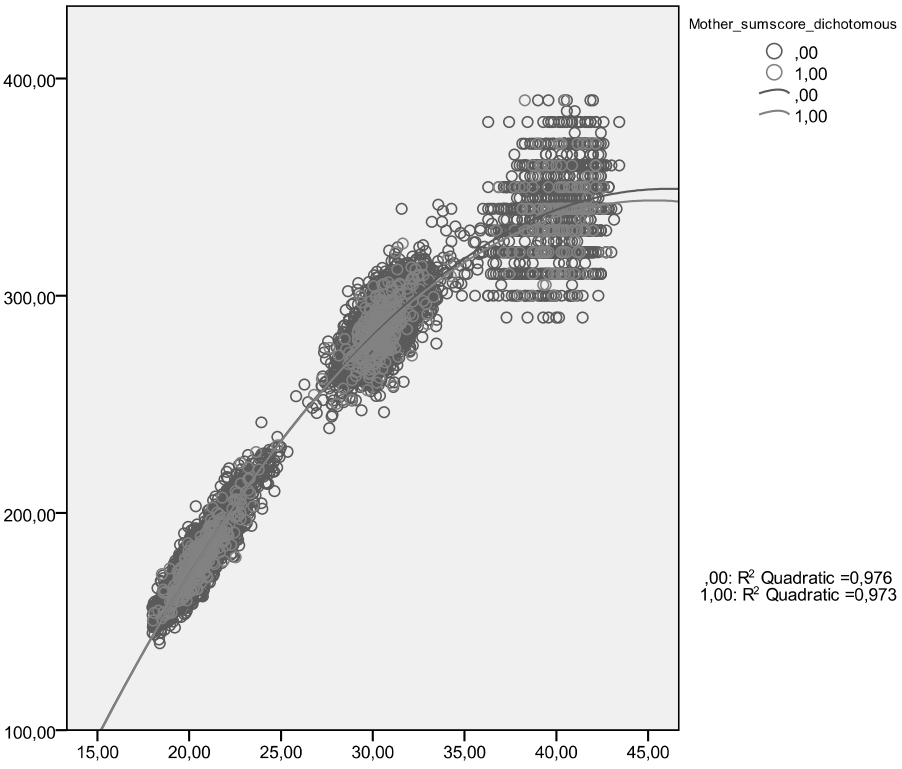
	MI 1	MI 2	MI 3	MI 4	MI 5	Pooled adjusted estimate	p-value
Exposure to:							
PAH	-0.00324	-0.00309	-0.00306	-0.00326	-0.00305	-0.003139	0.7583
Pesticides	-0.03473	-0.03537	-0.03497	-0.03521	-0.03507	-0.035071	0.0289
Phthalates	-0.01803	-0.01825	-0.01810	-0.01826	-0.01827	-0.018183	0.0452
Organic solvents	-0.00697	-0.00716	-0.00694	-0.00702	-0.00714	-0.007048	0.1763
Alkylphenolic compounds	-0.00889	-0.00918	-0.00887	-0.00894	-0.00906	-0.008990	0.1479
Metals	-0.01206	-0.01239	-0.01200	-0.01221	-0.01220	-0.012172	0.2629
Any chemicals	-0.00479	-0.00494	-0.00476	-0.00487	-0.00489	-0.004850	0.2796

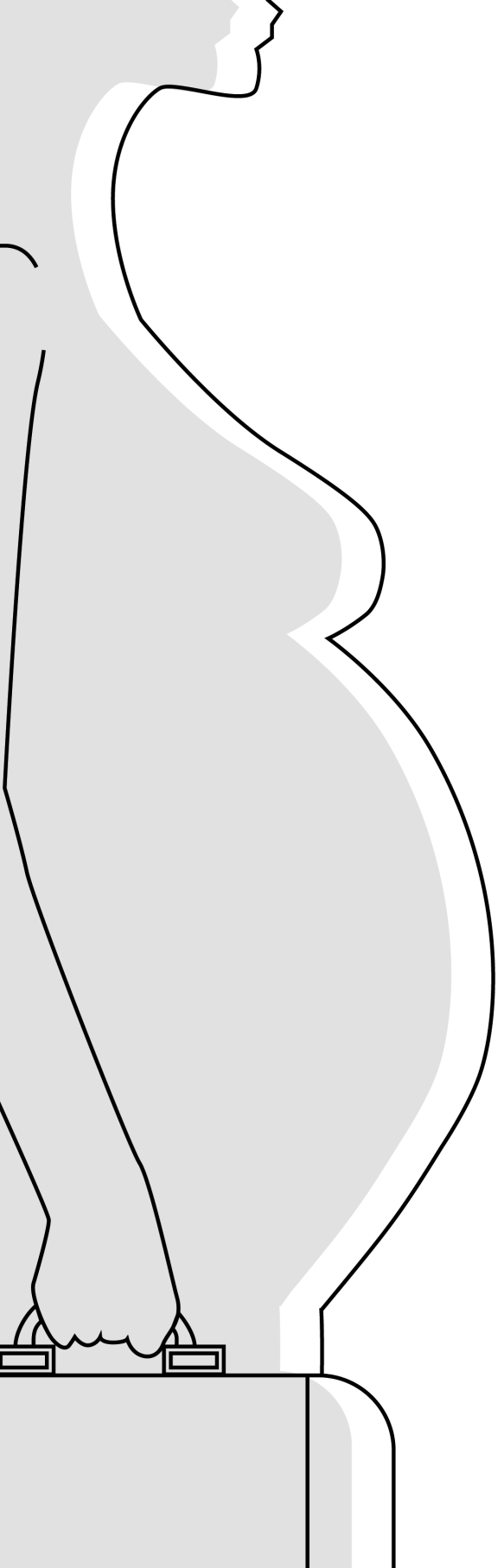
Abbreviations: MI=multiple imputation set. Estimates are adjusted for the following confounders: maternal age, educational level, ethnicity, foetal gender, weight before pregnancy, height at intake, smoking during pregnancy, alcohol use during pregnancy, folic acid use, parity, long periods of standing, lifting >5 kg at work, lifting >25kg at work, night shifts, preeclampsia, pregnancy induced hypertension and diabetes gravidarum.

SUPPLEMENT 2. Estimated foetal weight in the second and third trimester, and birth weight (in grams) plotted against gestational age in weeks. The two lines resemble a quadratic line, fitted at the points of women exposed to any of the chemicals, compared to non-exposed women.



SUPPLEMENT 3. Head circumference in the second trimester, third trimester, and at birth (in mm) plotted against gestational age in weeks. The two lines resemble a quadratic line, fitted at the points of women exposed to any of the chemicals, compared to non-exposed women.





Chapter 2.4

Bisphenol A and foetal growth

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ABSTRACT

Background: Prenatal exposure to Bisphenol A (BPA) has been associated with adverse birth outcomes.

Objective: We investigate the relation of prenatal BPA exposure with intrauterine growth and evaluate the effect of the measurement strategy on observed associations.

Methods: This study was embedded in a Dutch population-based prospective cohort study, with urine samples collected during early, mid, and late pregnancy. The study comprised 219 women, of which 99 had one measurement, 40 had two measurements, and 80 had three measurements of urinary BPA. Foetal growth characteristics were repeatedly measured by ultrasound during pregnancy and combined with measurements at birth. Linear regression models for repeated measurements of both BPA and foetal growth were used to study associations between log-transformed urinary concentrations of creatinine-based BPA ($lnBPA_{CB}$) and intrauterine growth.

Results: The relationship between BPA_{CB} and foetal growth was sensitive to the number of BPA measurements per woman. Among women with three BPA measurements, women with BPA_{CB} > 4.22 µg/g Crea relative to women with BPA_{CB} <1.54 µg/g Crea had lower growth rates for foetal weight and head circumference, resulting in a difference at birth of 3.9 cm (11.5% of mean) in head circumference and 683 grams (20.3% of mean) in birth weight. When fewer measurements were available per woman, the exposure-response relationship became progressively attenuated and statistically non-significant.

Conclusion: In our study population findings are compatible with observations that higher concentrations of urinary BPA are inversely associated with foetal growth. Further evidence is needed to corroborate these findings to the general population.

INTRODUCTION

Pregnant women are exposed to a variety of chemicals during pregnancy,[1,2] which may increase the risk of adverse health outcomes.[3] Environmental exposures that have been associated with adverse foetal development are heavy metals,[4,5] phthalates,[6] and pesticides.[7,8]

BPA is used to make polycarbonate polymers and epoxy resins, along with other raw materials in plastics production, and is present in dental fillings, plastic food and water containers, baby bottles, food wraps, as well as in the lining of beverage and food cans, presenting a large number of opportunities for human exposure.[9-11] Given the ubiquity of BPA in the human environment, exposure to BPA is virtually universal.[2] BPA is known to exert estrogenic activity and is considered an endocrine disrupting chemical (EDC).[12] Concern about EDCs stems from their potential effects via diverse mechanisms, including estrogenic/antiandrogenic properties, antioxidant actions, inhibition of cell cycles, and cell differentiation.[13,14] Some animal studies showed that exposure to EDCs mimicking sex steroids/steroids affected foetal growth and organ differentiation.[15,16]

Animal studies have shown that BPA reduced sperm quality, disturbed hormonal balance, and caused reproductive organ damage and malformations.[17-19] In rats, prenatal exposure to BPA led to both a reduction as well as a gain in body weight.[16,20] Recently, several epidemiological studies have considered the effects of prenatal exposure to BPA on reproductive health. Miao et al. found that job-related maternal exposure to BPA during the index pregnancy was associated with a decreased birth weight.[21] Lee and colleagues reported among 125 pregnant women, that maternal BPA levels in urine during the first trimester were inversely correlated with head circumference in the third trimester.[22] In contrast, Wolff et al. suggested that higher urinary BPA concentrations in the third trimester of pregnancy were associated with slightly higher birth weight of the offspring[23] and Philippat et al. showed an increase in head circumference with increasing BPA concentrations.[24]

The limited, contradictory findings in epidemiological studies on effects of BPA on foetal weight and birth weight might be associated with methodological issues related to exposure assessment. Pharmacokinetic studies suggest that BPA is rapidly metabolised with a short half life, resulting in low to modest correlations between repeated BPA measurements over 1-6 month periods.[25,26] A recent study by Braun et al. reported an intraclass correlation coefficient of 0.11 for BPA across three repeated urine samples during pregnancy, illustrating the need for repeated urinary BPA measurements during pregnancy in order to obtain sufficiently precise exposure estimates.[27]

With this study we aimed to investigate the effects of prenatal exposure to BPA on intrauterine growth and to evaluate the effects of the measurement strategy chosen on the observed associations.

MATERIALS AND METHODS

Study design

The Generation R Study is a population-based prospective cohort study on growth, development, and health from early foetal life until young adulthood in Rotterdam, the Netherlands.[28] All pregnant women with an expected delivery date between April 2002 and January 2006 in the study area of Rotterdam were invited to participate. In total, 9778 pregnant women (response 61%) participated in the study of which 8880 women enrolled during pregnancy and another 898 women at birth of their child. Extensive assessments were carried out during early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18-25), and late pregnancy (gestational age > 25 weeks), including biological samples. The study was approved by the Medical Ethics Committee at Erasmus University Medical Centre Rotterdam, The Netherlands (MEC 198.782/2001/31). Written informed consent was obtained from all participants.

Urine collection and analysis

In 2006 among all women who provided one urine sample, a random sample of 100 women was taken and analysed for organophosphorous pesticide, BPA, and phthalate levels.[1] In 2010 among all women with multiple urine samples available, a random sample was taken of 120 women, consisting of 40 women with two samples and 80 women with three samples. After exclusion of one twin pregnancy, a total of 219 women with 419 urine samples were available, with 26% in the first trimester, 28% in the second trimester, and 46% in the third trimester of pregnancy. All urine samples (65 ml) were collected between February 2004 and November 2005. All samples were taken between 8 a.m. and 8 p.m. in 100 ml polypropylene urine collection containers that were kept maximally 20 hours in a cold room (4°C) before being frozen in 20 ml portions in 25 ml polypropylene vials at -20°C. The urine specimens were analysed for BPA using tandem mass spectrometry. For the 100 specimens analysed in 2006, this was done at the Institute of Occupational, Social, and Environmental Medicine of the University of Erlangen-Nurnberg, Germany.[1] The 120 specimens in 2010 were analysed at the Institute of Prevention and Occupational Medicine, German Social Accident Insurance, Institute of the Ruhr-Universitat, Bochum, Germany (IPA).[29] To determine BPA, analytes were hydrolyzed and separated from 1 ml of urine using semi-automated steam distillation and solid-phase extraction. In Erlangen, the limit of detection (LOD) was 0.26 µg/L; at IPA the LOD was 0.05 µg/L. The between assay coefficient of variation was 8.3% in Erlangen and 5.6% at IPA. The within assay variability was between 3.4 and 6.5%. In Erlangen, derivatisation into tert-butyldimethylsilyl was needed, while in IPA, due to improvements in measurement method, no derivatisation was needed, which minimised the influence of BPA contamination due to sample workup, thus also allowing a lower LOD. Urinary creatinine concentrations were determined by the method described by Larsen.[30]

Foetal growth and birth outcomes

We combined second- and third trimester foetal ultrasound measurements with measurements of foetal size at birth. We measured growth characteristics to the nearest millimeter using standardised ultrasound procedures in the second (median 20.5, minimum-maximum 18.2-25.0 weeks) and third (median 30.2, minimum-maximum 27.4-33.8 weeks) trimester. First trimester measurements were used to establish gestational age, since use of the last menstrual period for pregnancy dating has several limitations,[31] and most women (76%) in our study population did not know the exact date of their last menstrual period. We used crown-rump length for pregnancy dating until a gestational age of 12 weeks and bi-parietal diameter for pregnancy dating thereafter in all women.[32,33] Estimated foetal weight (EFW) was calculated using the formula by Hadlock et al.[34] The intraclass correlation coefficient of foetal growth measurements was 0.95, based on 21 subjects, indicating a strong relation for different foetal biometry measurements between and among observers.[35] Internal reference curves were made for foetal weight and foetal head circumference during pregnancy, showing typical parabolic patterns. For all growth characteristics in the second and third trimester standard deviation scores (SD), based on the whole Generation R cohort, were constructed.[31] This method closely resembles the commonly used z-scores approach suggested by the World Health Organisation.[36] Information about gestational age, gender, weight, length, and head circumference at birth was obtained from medical records and hospital registries. For almost all women (n = 217, 99.1%) two measures of foetal growth were available, of which 157 women (72%) had complete information on all three measurements.

Potential confounders

The following well-known determinants of foetal growth were included as confounders in the association between urinary BPA and foetal growth: maternal age, pre-pregnancy weight, height, educational level, ethnicity, parity, smoking, alcohol use, and folic acid supplement use. Maternal height was measured at intake in the study. Maternal age, educational level, ethnicity, parity, and folic acid supplement use were obtained by questionnaire at enrolment in the study. Maternal smoking habits and alcohol use were assessed by questionnaire in each trimester and classified as abstainer, user until pregnancy was known, or user during pregnancy.

Statistical analyses

The distributions of urinary BPA concentrations were highly skewed and therefore all values were log transformed ($lnBPA_{CB}$) in order to obtain normal distributions. Women with a value below the LOD, were imputed with LOD/sqrt(2). Repeated measurement analyses were conducted with the Proc Mixed module of the Statistical Analysis System (version 9.2; SAS Institute Inc, Cary NC). First, a mixed effect model was used with $lnBPA_{CB}$ as dependent variable in order to assess which time-independent maternal characteristics and lifestyle factors influenced BPA concentrations, taking into account random variation within and between subjects in BPA

concentrations. Second, mixed effect models were used with repeated measurements of foetal head circumference and foetal weight as dependent variables and $lnBPA_{CB}$ as independent variable measured in the previous trimester. Thus, measurements of $lnBPA_{CB}$ in urine samples at first, second, and third trimester during pregnancy were related to foetal growth measurements at second and third trimester during pregnancy and at birth.

In these regression models, we used standard deviation (SD) scores as parameter of foetal growth (dependent variable). We used both $lnBPA_{CB}$ as continuous variable and $lnBPA_{CB}$ categorised into quartiles, based on distribution of BPA concentrations in all 219 women. This comparison allowed us to examine the shape of the relationship between $lnBPA_{CB}$ and foetal growth. The final model can be written as (for example for foetal weight): SD score of foetal weight = $ß_0$ + $ß_1$ x GA [gestational age [in weeks]] + $ß_2$ x quartiles $lnBPA_{CB}$ + $ß_3$ x GA x quartiles $lnBPA_{CB}$.[37] In this model, the coefficient $ß_3$ reflects the slope (interaction of $lnBPA_{CB}$ with gestational age), and tests whether foetuseses of women in the highest quartiles of $lnBPA_{CB}$ concentrations grow at the same rate as the foetuseses of women in the lowest quartile of $lnBPA_{CB}$ concentrations. The latter coefficient expresses a higher or lower foetal growth rate per week (in change SD score per gestational week) in exposed groups relative to the reference group with the lowest $lnBPA_{CB}$ concentration. The regression models were adjusted for all potential confounders.

Missing values in lifestyle and socioeconomic confounders were handled by multiple imputations (fully conditional specification, Markov Chain Monte Carlo method) by generating five independent datasets for all analyses, using SPSS version 17.0 for windows. All variables in Table 1 were included in the imputation procedure (these variables were imputed and used as predictor).

The influence of the availability of measurement information on the observed exposure-response relationship was evaluated by comparing three approaches. In the first approach it was assumed that only a single BPA measurement was available per woman. For women with multiple measurements a random selection procedure was used to assign a single measurement to each woman, resulting in a study sample of 219 women to study the association between a single $lnBPA_{CB}$ measurement and measures of foetal growth across pregnancy periods. In the second approach the study sample was limited to 120 women with at least two $lnBPA_{CB}$ measurements available. Again a random selection procedure was used to select two measurements among those women with three measurements. In the third approach the study sample was further restricted to the 80 women with three $lnBPA_{CB}$ measurements available across every trimester. For each study sample a similar regression model was used to directly compare exposure-response relationships among all approaches. In a sensitivity analysis we applied these three approaches among the 80 women with complete information, in order to eliminate potential effects of selective participation in the urine sample procedures which might have biased the analyses with different numbers of women included.

TABLE 1. Baseline characteristics of all women with at least one available BPA measurement (n=219) participating in the Generation R cohort

Maternal characteristics	Results
Age at intake in years, mean +/- SD	30.8 ± 5.2
Weight before pregnancy in kilograms, median (interquartile range)	63.0 (15.3)
Height measured at intake in centimeters, median (interquartile range)	168.0 (11.0)
Educational level	
Low	39 (17.8%)
Mid-low	56 (25.6%)
Mid-high	55 (25.1%)
High	50 (22.8%)
Missing	19 (8.7%)
Ethnicity	
Dutch	120 (54.8%)
Surinamese and Dutch Antilleans	19 (8.7%)
Moroccan and Turkish	29 (13.2%)
Other	34 (15.5%)
Missing	17 (7.8%)
Parity	
Nulliparous	112 (51.1%)
Multiparous	99 (45.2%)
Missing	8 (3.7%)
Smoking	
Yes, during pregnancy	27 (12.3%)
Yes, until pregnancy was known	10 (4.6%)
No	158 (72.1%)
Missing	24 (11.0%)
Alcohol	
Yes, during pregnancy	74 (33.8%)
Yes, until pregnancy was known	28 (12.8%)
No	92 (42.0%)
Missing	25 (11.4%)
Folic acid use	
No	40 (18.3%)
Yes, post conception start	49 (22.4%)
Yes, preconception start	82 (37.4%)
Missing	48 (21.9%)
Birth outcomes	
Gestational age (GA) at birth in weeks, median (interquartile range)	40.00 (2.00)
Birth weight in grams, mean +/- SD	3372.28 ± 589.14
Male	105 (47.9%)
Head circumference at birth in centimeters, mean +/- SD	33.84 ± 1.49
Length at birth in centimeters, mean +/- SD	50.14 ± 2.17
First trimester GA at urine collection in weeks, mean +/- SD	13.24 ± 1.74
Second trimester GA at urine collection in weeks, mean +/- SD	20.67 ± 1.12
Third trimester GA at urine collection in weeks, mean +/- SD	30.37 ± 1.53
Urine creatinine in grams/Litre, median (interquartile range)	0.69 (0.66)

Values are absolute numbers (percentages) for categorical variables unless otherwise indicated.

In a second sensitivity analysis we investigated whether the adjustment for creatinine could influence the results. In this analysis lnBPA levels were used as an independent variable, with creatinine levels as an adjustment factor in the models. A third sensitivity analysis investigated whether the time lag model used in this study could influence our results, since within person variability in BPA is high. Measurements of $lnBPA_{CB}$ in urine samples at first, second, and third trimester during pregnancy were related to foetal growth measurements at first, second, and third trimester during pregnancy.

RESULTS

Table 1 shows the baseline characteristics of the study population. The mean age of the women at intake in the study was 30.8 years. Of all women, 22.8% had completed higher education and the largest group was from Dutch origin (54.8%). The majority of women were nulliparous (51.1%). A total of 12.3% of the mothers continued smoking and 33.8% of the mothers continued drinking alcohol after the pregnancy was known.

There were no significant differences between the Bisphenol A (BPA) concentrations in the first, second, and third trimester of pregnancy nor for the analytical procedure by year. Furthermore, there were no differences in BPA concentration in different trimesters, or overall, by site (Erlangen or IPA) of analysis. BPA and creatinine-based BPA concentrations in three trimesters of pregnancy stratified by year of analyses are shown in Supplement 1. A lower educational level and being from Moroccan or Turkish origin was associated with a lower creatinine-based lnBPA ($lnBPA_{CB)}$. Alcohol use during pregnancy was associated with a higher $lnBPA_{CB}$ (Table 2).

Table 3 shows the univariable and multivariable linear analyses of $lnBPA_{CB}$ on foetal weight and foetal head circumference, using all 419 measurements from 219 women. The covariates did not confound the relation between BPA concentrations and foetal growth, since the effect estimates from the univariable and multivariable models were largely comparable (Supplement 2). However, since these covariates are important determinants of foetal growth, we included these covariates by default in all further multivariable models. When comparing women included in our study population to the women who provided urine and women in the whole Generation R cohort, we noticed that the women in our sample were slightly higher educated, more often of Dutch origin, and more often multiparous (Supplement 3).

Table 4 shows the influence of the number of BPA measurements on growth rates for foetal weight and foetal head circumference. Among the 80 women with three BPA measurements significantly lower growth rates were observed for both foetal weight and foetal head circumference. Foetuseses of women with higher exposure levels showed decreased foetal growth, but a significant trend across exposure groups was not observed. When fewer measurements were available per pregnant woman, the exposure-response relationship became progressively attenuated and statistically non-significant. The effect estimates of the univariable and

TABLE 2. Determinants of urinary BPA concentrations in 219 pregnant women participating in the Generation R Cohort

Variables	n	Intercept coefficient	Regression coefficient Change in $lnBPA_{CB}$	% change in BPA_{CB}
Educational level		1.25		
Low	39		-0.37 (-0.70, -0.03)*	-30.59%*
Mid-low	56		-0.29 (-0.58, 0.00)	-25.11%
Mid-high	55		-0.31 (-0.58, -0.03)*	-26.35%*
High	50		Reference	Reference
Ethnicity		1.05		
Dutch	120		Reference	Reference
Surinamese and Dutch Antilleans	19		-0.00 (-0.38, 0.37)	-0.42%
Moroccan and Turkish	29		-0.43 (-0.79, -0.07)*	-34.87%*
Other	34		0.19 (-0.11, 0.49)	21.41%
Parity		1.10		
Nulliparous	112		Reference	Reference
Multiparous	99		-0.14 (-0.35, 0.07)	-12.80%
Smoking		1.05		
No	158		Reference	Reference
Yes, until pregnancy was known	10		-0.21 (-0.69, 0.28)	-18.72%
Yes, during pregnancy	27		-0.06 (-0.44, 0.31)	-6.17%
Alcohol		0.86		
No	92		Reference	Reference
Yes, until pregnancy was known	28		0.39 (0.06, 0.72)*	47.45%*
Yes, during pregnancy	74		0.22 (-0.02, 0.47)	24.87%
Folic acid supplement use		1.01		
No	40		-0.02 (-0.32, 0.29)	-1.84%
Yes, post conception start	49		0.08 (-0.19, 0.34)	7.80%
Yes, preconception start	82		Reference	Reference

$lnBPA_{CB}$ = log transformed creatinine based total BPA concentration.
* p-value < 0.05.

multivariable analyses in the restricted study sample were comparable, suggesting little influence of the potential confounders.

In the sensitivity analysis on the 80 women with three BPA measurements the range of confidence limits around the estimates decreased, the number of measurements per woman increased and, in general, the magnitude of the estimate increased (Supplement 4). These women with complete urine samples were more often highly educated (33.8% versus 22.8%) and of Dutch origin (61.3% versus 54.8%). We found comparable effect estimates for foetal growth parameters for both $lnBPA_{CB}$ as well as lnBPA with additional adjustment for creatinine

TABLE 3. Univariable and multivariable repeated linear regression analyses between prenatal exposure to BPA_{CB} and SD scores of foetal weight and foetal head circumference among 219 pregnant women

Variables	Foetal weight Unadjusted beta coefficient (95%CI)	Foetal weight Adjusted beta coefficient (95%CI)	Foetal head circumference Unadjusted beta coefficient (95%CI)	Foetal head circumference Adjusted beta coefficient (95%CI)
Foetal growth rates [a]				
BPA_{CB} (µg/g Crea)				
< 1.54	Reference	Reference	Reference	Reference
1.54 < BPA_{CB} < 2.51	-0.009 (-0.033, 0.014)	-0.010 (-0.033, 0.014)	-0.016 (-0.045, 0.013)	-0.018 (-0.047, 0.011)
2.51 < BPA_{CB} < 4.22	-0.018 (-0.041, 0.006)	-0.015 (-0.038, 0.009)	-0.019 (-0.047, 0.009)	-0.016 (-0.044, 0.013)
> 4.22	-0.001 (-0.024, 0.023)	0.001 (-0.023, 0.025)	-0.018 (-0.049, 0.012)	-0.016 (-0.047, 0.014)
Per unit increase in BPA_{CB}	-0.013 (-0.025, -0.001)*	-0.011 (-0.023, 0.002)	-0.005 (-0.020, 0.009)	-0.003 (-0.018, 0.011)

BPA_{CB} = creatinine based total BPA concentration, * p-value < 0.05.

[a] beta coefficient represents the average decline/increase in SD score of foetal weight or foetal head circumference per gestational week.

TABLE 4. Multiple repeated linear regression analyses of the relation between number of urine samples analysed for BPA and effect on foetal growth rates during pregnancy

Samples/women		Number of women	Foetal weight Beta coefficient (95%CI)	Foetal head circumference Beta coefficient (95%CI)
Three samples		80		
BPA_{CB} (µg/g Crea)	< 1.54		Ref	Ref
BPA_{CB} (µg/g Crea)	1.54 < BPA_{CB} < 2.51		-0.041 (-0.081, -0.001)*	-0.052 (-0.098, -0.006)*
BPA_{CB} (µg/g Crea)	2.51 < BPA_{CB} < 4.22		-0.043 (-0.082, -0.004)*	-0.046 (-0.090, -0.003)*
BPA_{CB} (µg/g Crea)	> 4.22		-0.029 (-0.070, 0.012)	-0.066 (-0.113, -0.019)*
BPA_{CB} (µg/g Crea)	Per unit increase in BPA_{CB}		-0.017 (-0.033, -0.001)*	-0.018 (-0.037, 0.000)+
Two samples		120		
BPA_{CB} (µg/g Crea)	< 1.54		Ref	Ref
BPA_{CB} (µg/g Crea)	1.54 < BPA_{CB} < 2.51		-0.018 (-0.045, 0.009)	-0.018 (-0.055, 0.018)
BPA_{CB} (µg/g Crea)	2.51 < BPA_{CB} < 4.22		-0.029 (-0.056, -0.003)*	-0.013 (-0.049, 0.022)
BPA_{CB} (µg/g Crea)	> 4.22		-0.003 (-0.033, 0.027)	-0.017 (-0.057, 0.023)
BPA_{CB} (µg/g Crea)	Per unit increase in BPA_{CB}		-0.008 (-0.024, 0.008)	-0.005 (-0.024, 0.013)
One sample		219		
BPA_{CB} (µg/g Crea)	< 1.54		Ref	Ref
BPA_{CB} (µg/g Crea)	1.54 < BPA_{CB} < 2.51		0.003 (-0.027, 0.032)	-0.011 (-0.049, 0.025)
BPA_{CB} (µg/g Crea)	2.51 < BPA_{CB} < 4.22		0.008 (-0.025, 0.040)	0.003 (-0.036, 0.041)
BPA_{CB} (µg/g Crea)	> 4.22		0.025 (-0.002, 0.052)	0.015 (-0.022, 0.051)
BPA_{CB} (µg/g Crea)	Per unit increase in BPA_{CB}		-0.007 (-0.023, 0.010)	0.011 (-0.008, 0.030)

BPA_{CB} = creatinine based total BPA concentration

* p-value <0.05, + p-value <0.10

Beta coefficient represents the average decrease in SD of foetal weight per gestational week.

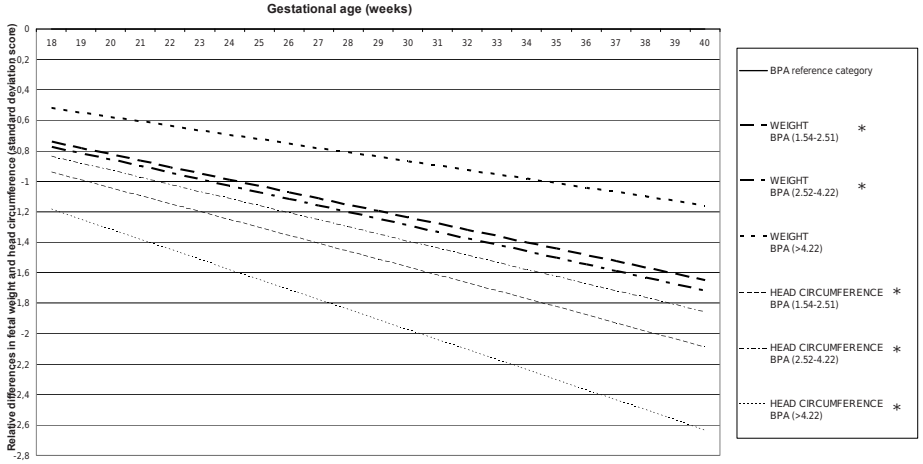
Adjusted for maternal age, educational level, ethnicity, parity, smoking during pregnancy, alcohol use during pregnancy, height at intake, weight before pregnancy, folic acid supplement use, and gender.

levels. Relating first, second, and third trimester BPA concentrations to first, second and third trimester foetal growth showed very similar results as the lagged model.

Figures 1 depicts the associations between $lnBPA_{CB}$ exposure categories and foetal weight and head circumference, based on the study population with three available BPA samples. Women in the highest BPA exposure group had the lowest growth rates for foetal head circumference, resulting in an average decrease of 2.63 SD at birth, which corresponds to approximately 3.9 cm (11.5%) of the average head circumference of 33.8 cm at birth. For foetal weight, women in the second highest exposure group showed an average decrease of 1.66 SD in birth weight, which corresponds to a difference of 683 grams (20.3%) at birth.

The within and between individual variance was 1.0728 and 0.4286, respectively, based on 120 women with more than one urine sample.

FIGURE 1. Relative differences in SD scores for foetal weight and head circumference in various lnBPACB exposure groups, compared to the lowest (<1.54) exposure group, among 80 women.



Adjusted relative differences in foetal weight and head circumference (SD scores) in the highest BPA exposure groups compared with the lowest exposure group. Values are based on repeated linear regression models and reflect the difference in the SD score of foetal weight or foetal head circumference measurements (based on 238 measurements for foetal weight, and 213 measurements for foetal head circumference) in the offspring of mothers in the highest BPA exposure groups compared with the offspring of mothers in the lowest exposure group. The reference value is a SD score of 0. * P-value < 0.05. Estimates are adjusted for the following confounders: maternal age, educational level, ethnicity, foetal gender, weight before pregnancy, height at intake, smoking during pregnancy, alcohol use during pregnancy, folic acid use, and parity.

DISCUSSION

The findings from this population-based prospective cohort study are compatible with the view that higher concentrations of creatinine-based Bisphenol A ($lnBPA_{CB}$) in prenatal urine are associated with lower foetal weight and head circumference. Furthermore, we demonstrated

that when three BPA measurements were used instead of a single BPA measurement, the associations between BPA and foetal growth were steeper and significant exposure-response estimates were obtained. In contrast, when three measurements were used, steeper and highly significant exposure-response estimates were obtained. Thus, increasing the number of measurements per subject during pregnancy seems to increase power of the study and result in less biased exposure-response estimates.

Epidemiological studies on the effects of prenatal BPA exposure on foetal development are rare. Lee et al. showed in 125 pregnant women that first trimester maternal urinary BPA levels negatively influenced foetal head circumference and abdominal circumference in the third trimester of pregnancy.[22] The present study corroborates these findings, although their effects on head circumference seem smaller. Furthermore, in a study among 587 children from families whereby occupational exposure to BPA of parents was ascertained through personal air sampling and exposure histories, prenatal exposure to BPA reduced birth weight, especially for maternal exposure.[21] Another study among 97 women showed that elevated prenatal BPA exposure, measured in maternal blood and umbilical cord blood, increased the risk of low birth weight, small-for-gestational-age, and adverse actions of adipokines in neonates.[38] In contrast, Wolff et al. suggested that higher urinary BPA concentrations in the third trimester of pregnancy were associated with slightly higher birth weight[23] and Philippat et al. showed an increase in head circumference with increasing BPA concentrations.[24]

Until recently, BPA was considered a weak environmental estrogen, about 10000 to 100000 fold less potent than estradiol.[39] However, studies on molecular mechanisms have revealed a variety of pathways through which BPA can stimulate cellular response at very low doses in addition to effects initiated by binding of BPA to the classical α- or more recent β-form of the estrogen receptor.[40] In humans, BPA is generally described as rapidly metabolised, with elimination thought to be virtually complete within 24 hours after exposure. Exposure is thought to be most exclusively from food, for example Wilson et al. estimated that 99% of exposure was of dietary origin, based on BPA measurements from a variety of sources such as food, air, and house dust.[41] However, a recent study by Stahlhut et al. reported that BPA levels did not decline rapidly with fasting time, which suggests substantial non-food exposure, or accumulation in body tissues such as fat.[42] Braun et al. observed numerous sources of BPA exposure during pregnancy, and recommended that epidemiological studies need to measure BPA concentrations more than once during pregnancy.[27]

When comparing the current levels of BPA in different trimesters of pregnancy in our study to other studies we may conclude that levels are very similar. For example, Braun et al. found GMs of BPA_{CB} of 1.7 (at 16 weeks), 2.0 (at 26 weeks), while our levels ranged between 1.7 and 3.3 (second and third trimester measurements).[27]

This study illustrates the profound influence of the chosen measurement strategy on the observed exposure-response associations. When using all available information on 219 women, no statistically significant associations were observed (see Table 3), whereas the analysis

restricted to 80 women with three BPA measurements clearly showed relevant associations (Table 4). These differences must be sought in two explanations. As mentioned before, women with complete information on BPA were more often of Dutch origin and highly educated, both determinants of higher BPA exposure. This restricted study population was more homogeneous for important determinants of exposure and, although the average exposure to BPA was higher the total variance in BPA was smaller. The observed within-individual variance reduced more than the between-individual variance compared with the full study sample. The latter will result in less biased estimates due to exposure variability. However, information on co-exposures, such as exposure to other endocrine disrupting chemicals was lacking. Braun et al. showed that BPA and phthalate concentrations were interrelated.[27]

A second explanation is that any exposure-response association will become attenuated when the exposure varies strongly over time and the relevant exposure to be considered is the long term average exposure. The attenuation depends on the ratio of the intra- and inter-individual variance in exposure and this attenuation may be counteracted by increasing the number of replicates per subject.[43] Based on a linear regression analysis with repeated exposure measurements and a continuous outcome measure, and the observed ratio of 2.5 for BPA measurements in our study population, increasing the number of measurements from 1 to 3 per person will reduce the attenuation from 70% to 45%. The BPA-foetal growth relation may fit the profile of a setting where, in a small study population, more replicates will maximise power.[44] This influence of the measurement strategy chosen might partly explain the lack of significant findings from some epidemiological and animal studies of BPA on foetal growth.[20,23,24]

We acknowledge that our study has several limitations, most importantly the small number of women in the analyses, and the lack of detail on which time window of exposure is biologically most relevant for foetal growth. In this study we used ultrasound measurements for pregnancy dating, and this appears to be superior to dating based on the last menstrual period.[31] A disadvantage is that growth variations in early pregnancy are assumed to be zero, impairing analyses on first trimester growth. The repeated measurements based on gestational age adjusted SD scores, comparable to standardised z-scores, enables us to identify pathological smallness rather than constitutional smallness. Foetal growth curves during pregnancy have a typical parabolic shape, which can be modeled by using fractional polynomials, but the advantage of SD scores is that growth can be analysed with a linear model.

The strength of this study is the population-based approach with recruitment during the prenatal period with multiple urine samples and a large number of potential confounders. Another strength of this study is the multiple observations on foetal growth as well as repeated measurements of BPA per subject, which will improve the precision of the analyses conducted. A limitation is the selective participation at baseline, with mothers of lower socioeconomic status less represented in the study population. However, we feel that selection had little influence on our results, since we randomly selected women from the study population, and exposure was ascertained independently from foetal growth characteristics.

Our results are compatible with the view that higher concentrations of BPA relative to lower concentrations of BPA in urine during pregnancy are associated with a decreased foetal growth for both foetal weight and head circumference. Furthermore, this study shows the influence of the measurement strategy chosen on the observed effect estimates, suggesting that in a small study population more replicates will maximise power. However, because of limitations of our study, we certainly need further evidence before we can conclude that in the general population BPA during pregnancy adversely influences foetal growth.

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SUPPLEMENT 1. Bisphenol A concentrations in three trimesters of pregnancy among 219 women participating in the Generation R cohort

BPA measurements	n (%)	LOD	DF (%)	GM	GSD	Min.	Percentile					Max.
							5th	25th	50th	75th	95th	
N=99 samples analysed in Erlangen												
Second trimester BPA (µg/l)	13 (13.1%)			1.3	3.5	<lod	<lod	1.0	1.3	3.8	8.0	8.0
Third trimester BPA (µg/l)	86 (86.8%)			1.1	3.9	<lod	<lod	0.5	1.2	2.5	9.5	46.0
Creatinine based second trimester BPA (µg/g Crea)	13 (13.1%)			1.9	2.5	0.4	0.4	1.0	1.9	4.4	8.7	8.7
Creatinine based third trimester BPA (µg/g Crea)	86 (86.8%)			1.7	2.9	0.1	0.3	0.8	1.5	3.9	8.4	22.8
All BPA concentrations (µg/g Crea)	99 (100%)	0.26	84.6	1.7	2.8	0.1	0.3	0.8	1.7	4.0	8.6	22.8
N=120 samples analysed in Bochum												
First trimester BPA (µg/l)	107 (33.4%)			1.3	3.4	0.1	0.2	0.6	1.1	3.3	10.9	24.6
Second trimester BPA (µg/l)	106 (33.1%)			1.7	3.2	0.2	0.3	0.7	1.7	3.1	13.8	64.8
Third trimester BPA (µg/l)	107 (33.4%)			1.6	3.3	0.2	0.2	0.8	1.5	2.9	17.3	87.2
Creatinine based first trimester BPA (µg/g Crea)	107 (33.4%)			3.1	2.1	0.7	0.9	1.9	3.0	4.9	11.6	23.2
Creatinine based second trimester BPA (µg/g Crea)	106 (33.1%)			3.3	2.3	0.8	1.0	1.9	2.8	4.2	23.4	40.4
Creatinine based third trimester BPA (µg/g Crea)	107 (33.4%)			3.2	2.6	0.6	0.9	1.7	2.5	4.2	34.1	172.0
All BPA concentrations (µg/g Crea)	320 (100%)	0.05	100.0	3.2	2.3	0.6	1.0	1.9	2.8	4.4	14.8	172.0

LOD: limit of detection, DF: detection frequency, GM=geometric mean, GSD=geometric standard deviation, Min: minimum, Max: maximum.

SUPPLEMENT 2. Overview of the covariates in the repeated linear regression analyses between prenatal exposure to $lnBPA_{CB}$ and SD scores of foetal weight and foetal head circumference among 219 pregnant women

Variables	Foetal weight Adjusted beta coefficient (95%CI)	Foetal head circumference Adjusted beta coefficient (95%CI)
Covariates [a]		
Maternal age (per year increase)	0.003 (-0.021, 0.026)	0.016 (-0.006, 0.038)
Pre-pregnancy weight (per kg increase)	0.004 (-0.006, 0.015)	0.003 (-0.006, 0.012)
Height at intake (per cm increase)	0.011 (-0.009, 0.031)	0.004 (-0.014, 0.022)
Gender (female)	0.053 (-0.150, 0.256)	-0.285 (-0.479, -0.091)*
Educational level		
Low	0.002 (-0.404, 0.407)	-0.294 (-0.660, 0.073)
Mid-low	-0.134 (-0.442, 0.173)	-0.043 (-0.342, 0.255)
Mid-high	-0.024 (-0.310, 0.263)	0.044 (-0.228, 0.315)
High	Reference	Reference
Ethnicity		
Dutch	Reference	Reference
Surinamese and Dutch Antilleans	-0.388 (-0.828, 0.052)	-0.145 (-0.521, 0.232)
Moroccan and Turkish	0.050 (-0.371, 0.471)	-0.001 (-0.393, 0.391)
Other	-0.085 (-0.408, 0.238)	-0.259 (-0.548, 0.029)
Parity		
Nulliparous	Reference	Reference
Multiparous	0.235 (0.006, 0.464)*	0.026 (-0.185, 0.238)
Smoking		
No	Reference	Reference
Yes, until pregnancy was known	0.188 (-0.249, 0.626)	0.271 (-0.166, 0.708)
Yes, during pregnancy	-0.226 (-0.544, 0.093)	-0.095 (-0.406, 0.216)
Alcohol		
No	Reference	Reference
Yes, until pregnancy was known	0.110 (-0.242, 0.462)	-0.054 (-0.381, 0.272)
Yes, during pregnancy	0.086 (-0.187, 0.359)	-0.038 (-0.286, 0.209)
Folic acid supplement use		
No	-0.123 (-0.411, 0.166)	-0.065 (-0.323, 0.194)
Yes, post conception start	-0.089 (-0.450, 0.271)	-0.162 (-0.521, 0.198)
Yes, preconception start	Reference	Reference

[a] beta coefficient is the overall effect of this covariate in the model in SD score of foetal weight or foetal head circumference.

SUPPLEMENT 3. Characteristics of participants in the BPA foetal growth subset, those with a complete set of pregnancy urine specimens, and the overall Generation R cohort

Maternal characteristics	BPA foetal growth subset (n=219)	Three urine specimens (n=2083)	Generation R Cohort (n=9778)
Age at intake (yr)	30.8 ± 5.2	29.3 ± 5.0	29.9 ± 5.4
Educational level			
Low	39 (17.8%)	459 (22.0%)	2270 (23.2%)
Mid-low	56 (25.6%)	591 (28.4%)	2628 (26.9%)
Mid-high	55 (25.1%)	426 (20.5%)	1655 (16.9%)
High	50 (22.8%)	488 (23.4%)	2006 (20.5%)
Missing	19 (8.7%)	119 (5.7%)	1219 (12.5%)
Ethnicity			
Dutch	120 (54.8%)	1009 (48.4%)	4443 (45.4%)
Surinamese and Dutch Antilleans	19 (8.7%)	224 (10.8%)	1055 (10.8%)
Moroccan and Turkish	29 (13.2%)	324 (15.6%)	1321 (13.5%)
Other	34 (15.5%)	443 (21.3%)	1931 (19.7%)
Missing	17 (7.8%)	83 (4.0%)	1028 (10.5%)
Parity			
Nulliparous	112 (51.1%)	1198 (57.5%)	5179 (53.0%)
Multiparous	99 (45.2%)	867 (41.6%)	4213 (43.1%)
Missing	8 (3.7%)	18 (0.9%)	386 (3.9%)
Smoking			
Yes, during pregnancy	27 (12.3%)	283 (13.6%)	1304 (13.3%)
Yes, until pregnancy was known	10 (4.6%)	171 (8.2%)	634 (6.5%)
No	158 (72.1%)	1398 (67.1%)	5656 (57.8%)
Missing	24 (11.0%)	231 (11.1%)	2184 (22.3%)
Alcohol			
Yes, during pregnancy	74 (33.8%)	666 (32.0%)	2786 (28.5%)
Yes, until pregnancy was known	28 (12.8%)	311 (14.9%)	1045 (10.7%)
No	92 (42.0%)	895 (43.0%)	3808 (38.9%)
Missing	25 (11.4%)	211 (10.1%)	2139 (21.9%)

Values are means ± standard deviation for normal distributed continuous variables and absolute numbers (percentages) for categorical variables.

SUPPLEMENT 4. Linear regression analyses for repeated measurements on the relation between number of urine samples analysed for BPA and effect on foetal growth rates during pregnancy

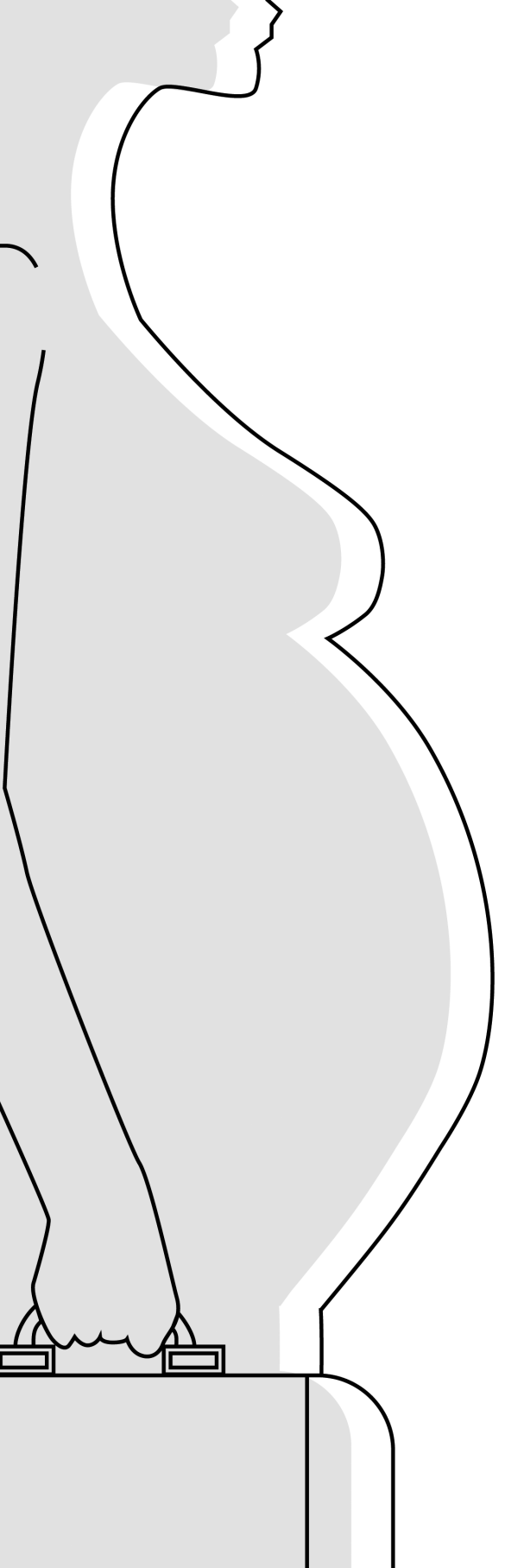
Samples/women		Number of women	Foetal weight Beta coefficient (95%CI)	Foetal head circumference Beta coefficient (95%CI)
Three samples		80		
BPA_{CB} (µg/g Crea)	< 1.54		Ref	Ref
BPA_{CB} (µg/g Crea)	1.54 < BPA_{CB} < 2.51		-0.041 (-0.081, -0.001)*	-0.052 (-0.098, -0.006)*
BPA_{CB} (µg/g Crea)	2.51 < BPA_{CB} < 4.22		-0.043 (-0.082, -0.004)*	-0.046 (-0.090, -0.003)*
BPA_{CB} (µg/g Crea)	> 4.22		-0.029 (-0.070, 0.012)	-0.066 (-0.113, -0.019)*
BPA_{CB} (µg/g Crea)	Per unit increase in BPA_{CB}		-0.017 (-0.033, -0.001)*	-0.018 (-0.037, 0.000)+
Two samples		80		
BPA_{CB} (µg/g Crea)	< 1.54		Ref	Ref
BPA_{CB} (µg/g Crea)	1.54 < BPA_{CB} < 2.51		-0.031 (-0.068, 0.006)	-0.044 (-0.085, -0.002)*
BPA_{CB} (µg/g Crea)	2.51 < BPA_{CB} < 4.22		-0.011 (-0.048, 0.026)	-0.035 (-0.076, 0.005)
BPA_{CB} (µg/g Crea)	> 4.22		-0.006 (-0.044, 0.031)	-0.065 (-0.111, -0.020)*
BPA_{CB} (µg/g Crea)	Per unit increase in BPA_{CB}		-0.016 (-0.036, 0.004)	-0.022 (-0.047, 0.004)+
One sample		80		
BPA_{CB} (µg/g Crea)	< 1.54		Ref	Ref
BPA_{CB} (µg/g Crea)	1.54 < BPA_{CB} < 2.51		-0.007 (-0.057, 0.044)	0.012 (-0.043, 0.068)
BPA_{CB} (µg/g Crea)	2.51 < BPA_{CB} < 4.22		0.015 (-0.035, 0.065)	0.015 (-0.045, 0.075)
BPA_{CB} (µg/g Crea)	> 4.22		-0.030 (-0.081, 0.022)	0.022 (-0.040, 0.085)
BPA_{CB} (µg/g Crea)	Per unit increase in BPA_{CB}		-0.027 (-0.065, 0.010)	0.005 (-0.035, 0.045)

BPA_{CB} = creatinine based total BPA concentration

* P-value <0.05, + P-value <0.10

Beta coefficient represents the average decrease in SD of foetal weight per gestational week.

Adjusted for maternal age, educational level, ethnicity, parity, smoking during pregnancy, alcohol use during pregnancy, height at intake, weight before pregnancy, folic acid supplement use, and gender.



Chapter 2.5

Chemicals and congenital heart defects

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ABSTRACT

Background: Congenital heart defects (CHDs) are the most common major malformations in newborns. In this study we examined the associations between the occurrence of CHDs in children and periconceptional occupational parental exposures to chemicals.

Methods: In an age-matched case-control study with standardised data collection at 15 months after birth, 424 mothers and 421 fathers of a child with CHD, and 480 mothers and 477 fathers of a non-malformed child, filled out questionnaires on periconceptional general and job characteristics. A job-exposure-matrix, which links the information on job title and a description of work tasks to an expert judgement on exposure to chemicals in the workplace, was used.

Results: The overall prevalence of occupational exposure to chemicals was 5.0% in cases and 6.2% in controls for mothers (Odds ratio (OR) adjusted = 0.92; 95% confidence interval (CI) 0.26-3.25), while 22.3% and 15.9% for fathers, respectively (ORadjusted = 1.23; 95%CI 0.39-3.91). No association of maternal occupational exposure to chemicals with risk of CHDs was found. Paternal exposure to phthalates was associated with a higher incidence of CHDs in general (ORadjusted = 2.08; 95%CI 1.27-3.40). Paternal exposure to phthalates was associated with peri-membranous ventricular septal defect (ORadjusted = 2.84; 95%CI 1.37-5.92), to polychlorinated compounds with atrioventricular septal defect (ORadjusted = 4.22; 95%CI 1.23-14.42) and to alkylphenolic compounds with coarctation of the aorta (ORadjusted = 3.85; 95%CI 1.17-12.67).

Conclusions: Periconceptional paternal (but not maternal) occupational exposure to chemicals is associated with an increased risk of CHDs in children. The results, however, must be interpreted cautiously as exposure probabilities are a crude measure of exposure.

INTRODUCTION

Congenital heart defects (CHDs) constitute the largest group of congenital anomalies, accounting for nearly 30% of children with major congenital anomalies diagnosed prenatally or in infancy in Europe.[1] The worldwide prevalence ranges from 6 to 8 per 1000 live births, and CHDs are associated with a high morbidity and mortality rate.[2] Risk factors, such as socio-economic status, maternal infections[3] and other environmental factors such as chemical exposure, have been associated with CHDs in epidemiological research.[4] Approximately 15% of CHDs can be attributed to known causes and 85% is related to interactions between subtle genetic variations and environmental exposures that interfere with embryonic cardiogenesis.[5]

Women constitute a substantial part of the labour force in Europe, and their participation is increasing, from 54% in 2002 to 58% in 2010 for women aged between 15 and 64 years.[6] With this increase in labour force participation, the likelihood that these women will be exposed to a variety of chemical, physical and psychological risk factors at work during pregnancy is also increased.[7] Women in paid employment generally have better pregnancy outcomes than those without paid jobs.[8,9] This is in contrast to women with a low socio-occupational status, which seemed to predispose to congenital anomalies of the respiratory, heart and circulatory systems.[10] Furthermore, certain work-related factors, such as exposure to chemicals,[11] physically demanding work[12] and psychological job strain,[13] may adversely influence pregnancy outcome. Thus, hazardous workplace conditions may have adverse effects on pregnancy outcome but have also been related to birth defects in the offspring.[14]

Occupational exposure to chemicals, especially during the periconceptional period, influences the reproductive system in both women and men and may lead to adverse health effects in children.[15] Studies on occupational exposure to chemicals or endocrine disrupting chemicals have shown associations with increased risks of congenital malformations, such as hypospadias and cryptorchidism, as well as a reduced sperm count.[16-21] Studies on certain occupations, such as hairdresser and laboratory workers, showed little evidence for associations with congenital malformations.[22-25] A review by Thulstrup and Bonde concluded that there is limited evidence linking occupational exposures during pregnancy to birth defects.[26] Epidemiological evidence of associations between occupational exposure to chemicals and CHDs is scarce and contradictory.[27-30] Prospective cohort studies on these associations are difficult because of the low prevalence of CHDs in the general population, requiring large sample sizes. Therefore, case-control studies with standardised postnatal data collection are the best alternative.[31] Since biomonitoring of chemicals is expensive and often not available, the job-exposure-matrix (JEM) is a valuable tool for valid exposure assessment in studies on reproductive outcome after chemical exposure.[32] The aims of our study were: 1) to study associations between CHDs and periconceptional parental occupational exposure to chemicals, and 2) to study whether chemical exposure is associated with different phenotypes of CHD.

MATERIALS AND METHODS

Design and study population

The HAVEN study is a case-control family study, designed to investigate determinants in the pathogenesis and prevention of CHDs, and has been described in detail.[31,33,34] In summary, recruitment of case and control children took place between June 2003 and January 2010 and case children with CHD were enrolled with both parents from four university medical centres: the Erasmus Medical Centre in Rotterdam, Leiden University Medical Centre in Leiden, VU University Medical Centre in Amsterdam, and Amsterdam Medical Centre, the Netherlands. Healthy control children and both parents were enrolled in collaboration with the child health care centres of 'Thuiszorg Nieuwe Waterweg Noord' in the large urban region of Rotterdam as part of the Western area of the Netherlands. Thus, the domain population comprised case- and control children born from 2002 onwards, all living in the Western area of the Netherlands. 74.7% of the responders in the case families and 61.4% of the responders of the control families participated in the present study. We did not have permission from the medical ethical committee to collect data on non-responders and those who did not want to participate.

Children with CHD diagnosed in the first 15 months after birth by paediatric cardiologists were identified from the hospital registry and invited to participate. Diagnoses were confirmed by echocardiography and/or cardiac catheterisation and/or surgery. Healthy control children of a similar age to case children and without any major congenital malformation (ascertained in regular health checks by child physicians) and both parents were randomly selected from the medical records from child health centres and invited to participate. The age of the case and control children was matched based on frequencies within age categories. At the time of data collection, 15 months after delivery, case and control families visited the hospital for the standardised collection of information on general characteristics and outcomes. In the present study we included 424 case children with both parents and 480 control children with both parents resulting in a total of 904 sets of children and their parents. The cases and controls were not matched on characteristics other than age. The definition of CHD phenotypes was based on reported gene-environment interactions[35-37] in the aetiology i.e. perimembranous ventricular septal defect (pVSD, $n = 113$), Tetralogy of Fallot ($n = 52$), atrioventricular septal defect (AVSD, $n = 44$), coarctation of the aorta (CoA, $n = 44$), hypoplastic left heart syndrome (HLHS, $n = 20$), aortic valve stenosis ($n = 9$), pulmonary valve stenosis ($n = 63$), transposition of the great arteries (TGA, $n = 63$), and miscellaneous ($n = 16$). The Central Committee of Human Research in The Hague and the Medical Ethical Committees of the participating hospitals reviewed and approved the study protocol (CCMO07.1052/MA/P03.0200, approval date March 27, 2003; MEC212.508/2002/91, approval date April 16, 2002). Written informed consent was obtained from all participants.

Data collection

At the time of data collection, 15 months after the end of the index-pregnancy, questionnaires were filled out by the mother and the father at home. During the hospital visit the questionnaires were verified by a researcher. The periconceptional period was defined as four weeks prior to conception until eight weeks after conception. The questionnaires requested information on parental age, height and weight, educational level, ethnicity, cigarette smoking, folic acid, alcohol and medication use, gender of the child and family history of CHDs. Ethnicity and educational level were classified according to the definitions of Statistics Netherlands.[38] A positive family history of CHDs was defined by one or more CHDs in the family of the mother or father in the third degree or closer. The maternal use of folic acid in the periconceptional period was defined as the daily use of at least 400µg folic acid during the complete periconceptional period. Inconsistent users were classified as non-users. We defined cigarette, alcohol, and medication use as any use during the periconceptional period. Standardised anthropometric measurements were performed, including maternal weight and height.

Geographic variation

As an individual's residence might be associated with occupational opportunities, area of residence by city, zip code and street name were collated into a measure of urban density, based on a method called 'area address density', a unit of measurement used by the Statistics Netherlands from 2003 onwards. Five degrees of urban density were distinguished, namely: 1) very high density regions (> 2500 addresses per square kilometre), 2) high density regions (1500-2500 addresses per square kilometre) 3) moderate density regions (1000-1500 addresses per square kilometre) 4) low density regions (500-1000 addresses per square kilometre) and 5) very low density regions (<500 addresses per square kilometre).[39]

Job-exposure-matrix

In the questionnaire work status and occupation were ascertained. Work status was based on a single question on current economic status, and subjects with paid employment were asked to fill out an open question providing a description of the job. We assessed occupational exposure to chemicals by applying a JEM, with a focus on endocrine disrupting chemicals.[32] Job descriptions were coded into job titles by the Dutch Standard Classification of Occupations, and linked to the JEM, which was based on the judgement of occupational hygienists who estimated for particular jobs the likelihood of exposure to seven categories of chemicals, namely pesticides, polychlorinated compounds, phthalates, bisphenol A, alkylphenolic compounds, heavy metals and miscellaneous agents.

Exposure assessment by the JEM was blinded to the outcome, and blinded to participants. Six fathers were excluded from analysis because of incomplete answers on work status and job description. The JEM focusses on the most important chemicals with relevant exposures in the occupational setting. The occupational hygienists scored the probability of exposure to

each chemical group for all job titles, in three levels: 'unlikely'(0), 'possible'(1), and 'probable'(2). For this study we collate categories one and two into one category indicating the possible occurrence (yes/no) of exposure to chemicals. An overall classification of 'possible exposure to chemicals' was collated if one of the seven chemical exposure categories was scored as 'yes'. Different JEMs have been successfully used as a valuable tool for exposure assessment in epidemiological studies on the health risks of chemicals, with a focus on endocrine disruption.[9,17,40,41]

Statistical analysis

General characteristics of mothers, fathers and children were compared between the groups using Mann-Whitney U test for continuous variables and Chi-square test for dichotomous variables. We used logistic regression analyses to study the associations between occupational exposure to chemicals and CHDs. For mothers we selected the following potential confounders: maternal age, educational level, ethnicity, parity, CHD in family, periconceptional alcohol use, periconceptional medication use, periconceptional use of folic acid and urban density. For fathers we selected the following confounders: paternal age, educational level, ethnicity and urban density. In the univariable and multivariable logistic regression analyses, multiple comparisons are made, thus we applied a Bonferroni correction. The agreement between the various exposure categories was calculated by the weighted Cohen's kappa.[42] We performed statistical analysis using the Statistical Package for the Social Sciences version 15.0. Statistical significance was set at $P < 0.05$.

RESULTS

A total of 424 case and 480 control children and both parents (excluding three fathers from both case and control groups) were included. The general characteristics of the study populations are shown in Table 1. Cases showed a significantly lower birthweight after adjustment for gestational age, compared with control children. Case and control mothers were significantly different for ethnicity, parity, alcohol use and CHD in the family. The urban density was significantly different between the case and control families.

TABLE 1. General characteristics of the case families of a child with a congenital heart defect (CHD) and control families of a non-malformed child enrolled in the HAVEN study

Variables	Cases (n = 424)	Controls (n = 480)
Characteristics of children		
Age at intake (months)	14.81 (2.66)	15.47 (2.47)
Gender		
Male	238 (56.1%)	270 (56.2%)
Female	186 (43.9%)	210 (43.8%)
Birthweight adjusted for GA (median, min-max)	3252 (795-5150)	3512 (1625-5920)*

CHD		
Isolated	318 (75.0%)	-
Non-isolated	106 (25.0%)	-
Characteristics of mothers		
Age (years) at birth of index child (mean, SD)	31.8 (4.7)	31.0 (4.5)*
Educational level		
Low	111 (26.2%)	105 (21.9%)
Intermediate	177 (41.7%)	239 (49.8%)
High	136 (32.1%)	136 (28.3%)
Ethnicity		
European Dutch Native	369 (87.0%)	394 (82.1%)*
European Others	15 (3.5%)	14 (2.9%)
Non-European	40 (9.4%)	72 (15.0%)
Primipara[a]	182 (42.9%)	238 (49.6%)*
Mulitpara[a]	242 (57.1%)	241 (50.2%)
Pregnancy		
Spontaneous pregnancy (yes)[a]	400 (94.6%)	456 (95%)
Previous abortion (no)	305 (71.9%)	355 (74%)
BMI (kg/m^2)(median, min-max)[b]	24.4 (11.9-41.5)	24.3 (16.0-52.2)
CHD in family (yes)	29 (6.8%)	17 (3.5%)*
Periconceptional:		
Folic acid	220 (51.9%)	254 (52.9%)
Smoking	77 (18.2%)	101 (21.0%)
Alcohol	166 (39.2%)	155 (32.3%)*
Medication	84 (19.8%)	81 (16.9%)
Characteristics of fathers		
Age (years) at birth of index child (mean, SD)	34.4 (5.3)	34.0 (5.1)
Educational level		
Low	112 (26.6%)	128 (26.8%)
Intermediate	141 (33.5%)	195 (40.9%)
High	168 (39.9%)	154 (32.3%)
Ethnicity		
European Dutch Native	361 (85.8%)	400 (83.9%)
European Others	14 (3.3%)	7 (1.5%)
Non-European	46 (10.9%)	70 (14.6%)
CHD in family (yes)	22 (5.2%)	19 (3.9%)
Occupational and geographical characteristics		
Paid employment mother	310 (73.1%)	371 (77.3%)
Paid employment father	401 (94.6%)	451 (94.0%)
Urban density		
Very high density area	105 (24.8%)	240 (50.0%)*
High density area	117 (27.6%)	223 (46.5%)*
Moderate density area	80 (18.9%)	11 (2.3%)*
Low density area	68 (16.0%)	4 (0.8%)*
Very low density area	53 (12.5%)	2 (0.4%)*

Values are absolute numbers (percentages) unless otherwise indicated. a) Numbers do not add up owing to 1 missing value in each variable b) Numbers do not add up owing to 5 missing values in this variable.

* Significant p-value <0.05.

Mann-Whitney U test was used for continuous variables and chi-square test for dichotomous variables.

GA: gestational age.

Table 2 presents the associations between CHDs and periconceptional parental occupational exposures to chemicals. The overall prevalence of occupational exposure to chemicals for case mothers was 5.0% and 6.2% for control mothers, and for fathers these figures were 22.3%, and 15.9%, respectively. No association of maternal occupational exposure to chemicals with risk of CHDs was found. After adjusting for potential confounders, we found an association between paternal occupational exposure to phthalates (adjusted odds ratio (OR) = 2.08; 95% confidence interval (CI) 1.27-3.40) and CHDs. When maternal and paternal risk factors were adjusted for each other, the ORs remained largely the same.

TABLE 2. Associations between periconceptional parental occupational exposures and CHDs in the offspring

Job exposure matrix	**Exposure prevalence**		**CHDs**	
	Cases n=424 N (%)	**Controls n=480 N (%)**	**OR (95% CI) unadjusted**	**OR (95% CI) adjusted**
Mothers[a]				
Exposure to:				
Pesticides	6 (1.4%)	9 (1.9%)	0.75 (0.27-2.13)	0.25 (0.05-1.36)
Phthalates	15 (3.5%)	8 (1.7%)	2.16 (0.91-5.16)	1.95 (0.67-5.61)
Alkylphenolic compounds	16 (3.8%)	24 (5.0%)	0.75 (0.39-1.42)	0.45 (0.19-1.07)
Heavy metals	3 (0.7%)	4 (0.8%)	0.88 (0.19-3.81)	1.40 (0.29-6.74)
Any of these substances	21 (5.0%)	30 (6.2%)	0.78 (0.44-1.39)	0.92 (0.26-3.25)
	Cases n=421 N (%)	**Controls n=477 N (%)**	**OR (95% CI) unadjusted**	**OR (95% CI) adjusted**
Fathers[b]				
Exposure to:				
Pesticides	23 (5.5%)	19 (4.0%)	1.39 (0.75-2.60)	0.72 (0.31-1.67)
Polychlorinated compounds	37 (8.8%)	35 (7.3%)	1.22 (0.75-1.97)	1.72 (0.98-3.02)+
Phthalates	63 (15.0%)	45 (9.4%)	1.69 (1.12-2.54)*	2.08 (1.27-3.40)* #
Alkylphenolic compounds	40 (9.5%)	26 (5.5%)	1.82 (1.09-3.04)*	1.60 (0.85-2.99)
Heavy metals	25 (5.9%)	19 (4.0%)	1.52 (0.83-2.80)	1.47 (0.71-3.06)
Any of these substances	94 (22.3%)	76 (15.9%)	1.52 (1.08-2.12)*	1.23 (0.39-3.91)

a) adjusted for maternal age, educational level, ethnicity, parity, CHD in family, periconception alcohol use, periconception medication use, periconception folic acid use, urban density.
b) adjusted for paternal age, educational level, ethnicity, urban density.
* significant, p-value <0.05,
+ p-value <0.10 and >0.05,
# significant after Bonferroni correction p-value < 0.008
OR: odds ratio, CI: confidence interval.

TABLE 3. Associations between periconceptional paternal occupational exposure and the risk of separate CHD phenotypes in the offspring

Job exposure matrix	CHD phenotypes				
	pVSD (n=113)	TOF (n=52)	AVSD (n=44)	CoA (n=44)	TGA (n=63)
Fathers[a]					
Exposure to:					
Pesticides	1.35 (0.44-4.18)	1.46 (0.38-5.69)	0.38 (0.04-3.59)	1.19 (0.25-5.64)	1.00 (0.23-4.35)
Polychlorinated compounds	2.13 (0.91-5.00)+	2.45 (0.85-7.00)+	4.22 (1.23-14.42)*	1.09 (0.25-4.73)	0.94 (0.26-3.37)
Phthalates	2.84 (1.37-5.92)*	2.32 (0.93-5.76)+	3.21 (0.98-10.54)+	1.76 (0.57-5.46)	2.03 (0.76-5.45)
Alkylphenolic compounds	2.19 (0.89-5.36)+	2.31 (0.84-6.35)	1.16 (0.24-5.63)	3.85 (1.17-12.67)*	1.80 (0.50-6.55)
Heavy metals	1.97 (0.69-5.65)	2.71 (0.88-8.41)+	0.50 (0.04-5.93)	2.40 (0.60-9.60)	0.63 (0.12-3.50)

Data are OR (95% CI).
a) adjusted for paternal age, educational level, ethnicity, urban density.
* significant, p-value <0.05,
+ p-value<0.10 and >0.05,
# significant after Bonferroni correction p-value <0.002.
pVSD: perimembranous ventricular septal defect, TOF: Tetralogy of Fallot, AVSD: atrioventricular septal defect, CoA: coarctation of the aorta, TGA: transposition of the great arteries.

Table 3 presents the results of the multivariable analysis showing associations between paternal occupational exposure to chemicals and separate CHD phenotypes. Paternal occupational exposure to polychlorinated compounds was associated with AVSD (ORadjusted = 4.22; 95%CI 1.23-14.42), to phthalates was associated with pVSD (ORadjusted = 2.84; 95%CI 1.37-5.92) and to alkylphenolic compounds was associated with CoA (ORadjusted = 3.85; 95%CI 1.17-12.67).

Kappa values for maternal exposure to phthalates and alkylphenolic compounds, and paternal exposure to polychlorinated compounds and phthalates, were 0.66 and 0.72, respectively. When we adjusted the association between paternal phthalate exposure and CHDs for exposure to polychlorinated compounds, the OR changed to 2.39 (95%CI 1.12-5.09) (data not shown).

DISCUSSION

This age-matched case-control study suggests that periconceptional occupational exposure of the father-to-be to chemicals, in particular phthalates, is associated with an increased occurrence of CHDs. Periconceptional maternal occupational exposure to chemicals overall or to specific chemicals was not associated with CHDs in the offspring.

Exposure assessment in this study was based on job title and activities, provided by fathers and mothers separately. The questionnaire focussed on the periconceptional period, and although the questionnaire was filled out approximately 15 months after child birth, the researcher verified every answer in a personnel interview. Job characteristics were available in

99.9% of the parents, because work history in general is recalled quite easily. Recall bias is very unlikely, as we did not ask for specific exposures but only for a description of the job and, moreover, the JEM ensures that exposure is classified independently from the outcome i.e. CHD, and is blinded to participants. A limitation of a JEM is that it does not account for variability within job titles. We tried to reduce the misclassification by assessing exposure based on both job title and description of the work tasks. The outcome of this matrix, however, must be interpreted cautiously as exposure probabilities are only a crude measure of exposure.

Little is known about potentially harmful environmental factors in the aetiology of CHDs. Several other studies investigated associations between occupational hazards, including exposure to chemicals, and specific phenotypes of congenital malformations or congenital malformations as a group.[19,26] Some of these studies found indications for effects of chemicals on foetal development but the evidence remains equivocal. The evidence on CHDs in particular is scarce. The associations found in this study between the periconceptional paternal occupational exposure to chemicals and CHDs could possibly be linked to effects of these substances on semen quality. Several studies have shown the potential for preconception occupational exposure to chemical substances to reduce semen quality.[43,44] Chemical exposure might disturb the epigenetic programming during maturation of the sperm cells, which may result in derangements in imprinted genes in particular in embryonic tissue, which may subsequently lead to birth defects.[45-47] Maternal occupational exposure to chemicals might be harmful during both maturation of the oocyte and embryogenesis. Exposure to pesticides and bisphenol A have been shown to impair growth and development in laboratory animals and possibly in humans.[48,49]

In this case-control study we studied couples with a child with CHD at the time of data collection, at approximately 15 months after the index pregnancy. This is considered a methodological strength of this study because this standardised data collection reduces misclassification in the selection of children with and without CHD. Parents of children who were diagnosed with a CHD in the first 15 months after birth were invited to participate, ensuring that the majority of children with CHD are included in our study, as most congenital malformations are diagnosed in the first year of life.[50] Potential misclassification of control children cannot be ruled out completely because although these children underwent regular physical examinations, including cardiac auscultation, they did not undergo doppler echocardiography. Children who died because of the CHD before the age of 15 months are not included in the study population, which may have led to some selection in the severity of the included CHDs. Probably, this selection is not associated with exposure to chemicals, and when exposure would be associated with the severity of CHDs, the selection may have caused an underestimation of the observed effect estimates. The stratified analyses have to be interpreted with caution because of small numbers and multiple comparisons. After Bonferroni correction the association between phthalate exposure and CHDs remained significant but owing to the small numbers in the phe-

notype analyses, the association between phthalates and pVSD (p-value 0.005) is just above the Bonferroni corrected p-value of 0.002.

To study the effect of possible selective participation among cases and controls, we primarily looked at educational level as a modifier of the observed associations between exposure and outcome. This analysis showed that education did not influence the observed associations. In addition, we observed that educational level was significantly associated with occupational exposure to chemicals, which was independent of case or control status. Therefore, we conclude that it is not very likely that selective participation confounded our results.

The exposure to phthalates, polychlorinated compounds and alkylphenolic compounds in this study population was related to occupations such as painter, electrician, metalworker, woodworker and the agricultural and horticultural trades. As mothers and fathers working in a specific occupation can be exposed to multiple chemicals, we calculated agreement between the different exposure categories. For mothers we found good agreement between exposure to phthalates and alkylphenolic compounds (kappa = 0.66), as mothers are likely exposed to both substances within similar jobs. For fathers we also observed good agreement between exposure to polychlorinated compounds and phthalates (kappa = 0.72). When we adjusted the association between paternal phthalate exposure and CHDs for exposure to polychlorinated compounds, the OR changed to 2.39 (95%CI 1.12-5.09). Owing to the interrelationship among these exposure groups, we had limited power to disentangle the specific role of phthalates and polychlorinated compounds in the observed occurrence of CHDs. Background exposure to various chemicals through diet and environment may also occur. However, it is unlikely that background exposure with a high prevalence is associated with occupational exposure with a low prevalence. Thus, background exposure will most likely not confound the relation between occupational chemical exposure and congenital anomalies. Furthermore, the level of exposure to chemicals within occupations is generally much higher than background exposure through diet and environment.[51] We did not assess background exposure, which may have contributed to unexplained variance in our outcome CHD, and therefore residual confounding cannot be completely ruled out.

Despite the fact that we recruited controls from the same source population in the Netherlands as the cases, we acknowledge that the area from which the cases were sampled is larger than the region from which the controls were sampled. However, based on the characteristics of both areas and populations it is unlikely that this has resulted in selection bias. We did observe differences between cases and controls regarding urban density and as the degree of urbanisation is related to occupational opportunities, we corrected for the degree of urbanisation to reduce any potential differences in sampling. There were no significant differences in occupational exposure to chemicals across the different centres for case recruitment.

While in the past ten years our knowledge of genetic contributions to CHDs has increased,[52] only a minority can be attributed to heritable genetic defects.[28] A review by Kopf and Walker shows in animal studies that the developing cardiovascular system is sensitive to many

environmental pollutants, such as dioxins, polychlorinated biphenyls and some pesticides.[53] Reviews on the influence of parental occupational exposure on congenital malformations have identified a large number of potentially hazardous occupational exposures, most notably pesticides, organic solvents and heavy metals.[54,55] Previous studies in humans showed associations between maternal exposure to fungicides or organic solvents and TGA and HLHS in the offspring.[28,30] A study in Baltimore showed that the proportion of congenital malformations that could have been prevented by eliminating known environmental risk factors was small, suggesting that many other environmental risk factors remain unknown.[56] In future studies additional measurements of exposures through biomarkers in human tissues and fluids is recommended to give more precise information on the level of exposure to certain chemicals and their potential consequences for CHDs.[57,58]

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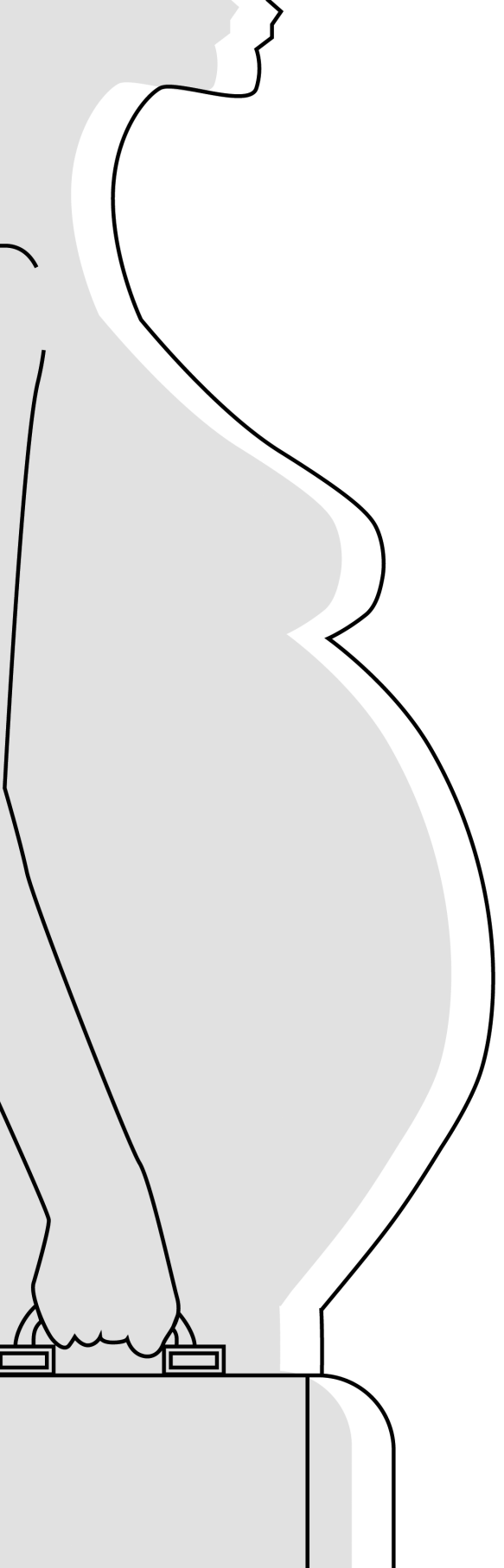
PART 3

PHYSICALLY DEMANDING WORK



Chapter 3.1

Physically demanding work, chemicals and hypertensive disorders during pregnancy

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ABSTRACT

Objective: To study the associations between physically demanding work and occupational exposure to chemicals and hypertensive disorders during pregnancy within a large birth cohort study, the Generation R Study.

Methods: Associations between occupational characteristics and hypertensive disorders during pregnancy were studied in 4465 pregnant woman participating in a population-based prospective cohort study from early pregnancy onwards in the Netherlands (2002-2006). Mothers who filled out a questionnaire during mid-pregnancy (response 77% of enrolment), were included if they conducted paid employment, had a spontaneously conceived singleton live born pregnancy, and did not suffer from pre-existing hypertension (n = 4465). Questions on physically demanding work were obtained from the Dutch Musculoskeletal Questionnaire and concerned questions on manually handling loads of 25 kg or more, long periods of standing or walking, night shifts, and working hours. To assess occupational exposure to chemicals, job titles and task descriptions were linked to a job-exposure-matrix (JEM), an expert judgement on exposure to chemicals at the workplace. Information on hypertensive disorders during pregnancy was obtained from medical records.

Results: We observed no consistent associations between any of the work-related risk factors, such as long periods of standing or walking, heavy lifting, night shifts, and working hours, nor exposure to chemicals with hypertensive disorders during pregnancy.

Conclusion: This prospective birth cohort study suggests that there is no association between physically demanding work and exposure to chemicals and hypertensive disorders during pregnancy. However, the low prevalence of pregnancy induced hypertension and preeclampsia, combined with the low prevalence of occupational risk factors limit the power for inference and larger studies are needed to corroborate of refute these findings.

INTRODUCTION

Hypertensive disorders during pregnancy are among the leading causes of maternal and neonatal morbidity worldwide, and include pregnancy induced hypertension (PIH) and preeclampsia.[1,2] PIH and preeclampsia complicate about 7% of all pregnancies[3] and severe preeclampsia is a major cause of severe maternal morbidity (e.g. stroke and liver rupture) and adverse perinatal outcomes, such as prematurity and intrauterine growth restriction.[4] Risk factors for PIH and preeclampsia include family or obstetric history of preeclampsia, first pregnancy, obesity, higher maternal age, pre-existing diabetes, renal disease, hypertension, and chronic autoimmune disease.[5-9] Evidence for the influence of environmental and occupational factors is contradictory to a few studies that have suggested that these factors may play a role in the aetiology of hypertensive disorders during pregnancy.[10] However, the underlying mechanisms for occupational risk factors, such as physically demanding work and exposure to chemicals, are unclear.

Physically demanding work, such as prolonged standing and frequent lifting, may increase catecholamine levels[11-13] which may affect constriction/dilatation of blood vessels.[14] High levels of catecholemines have been demonstrated in patients suffering from preeclampsia.[15] Furthermore, increased catecholamine levels are hypothesised to decrease uterine blood flow and may therefore influence early placentation.[12] Contradictory findings have been reported on physically demanding work and occurrence of PIH or preeclampsia. Mozurkewich et al. showed in a meta-analysis, based on four studies, that physically demanding work was significantly associated with PIH and preeclampsia (OR 1.60, 95%CI 1.30-1.96).[16] A more recent and larger review by Bonzini et al., based on eight studies, concluded that for preeclampsia and PIH, although several positive findings were reported, the evidence base was too limited to allow firm conclusions. This second review excluded less articles than Mozurkewich et al., and included five more years of research, covering almost twice the number of articles. No meta-analysis could be performed, due to the large heterogeneity in exposure definitions, and the available evidence was not sufficient to justify mandatory restrictions on any of the occupational activities during pregnancy.[17] This latter review included some new studies that showed modest to no effect of several aspects of physically demanding work, such as working hours, standing, lifting, physical activity, and shift work on PIH and preeclampsia.[18-23] However, a recent study by Haelterman et al., which is not included in either review, showed that prolonged standing increased the risk of preeclampsia.[24]

Occupational exposure to chemicals in relation to hypertensive disorders during pregnancy has been rarely studied. Some studies on maternal exposure to chemicals have suggested that organic solvents[25] and pesticides[26] may increase the risk of hypertensive disorders. Based on these previous studies, we hypothesised that occupational risk factors, such as physically demanding work and exposure to chemicals, may influence the occurrence of PIH or preeclampsia. Since studies on occupational risk factors showed conflicting results, it is unclear how working pregnant women should be managed. Further studies are needed to elucidate

the role of occupational risk factors in the pathogenesis of PIH and preeclampsia, so that preventive measures, if needed, can be taken.

The aim of this study was to assess, in a population-based prospective cohort study, the associations between physically demanding work and exposure to chemicals with hypertensive disorders during pregnancy.

MATERIAL AND METHODS

Design and study population

The Generation R Study is a population-based prospective cohort study on growth, development, and health from early foetal life until young adulthood in Rotterdam, The Netherlands. The study design has been described in detail previously.[27] Briefly, all pregnant women who had an expected delivery date between April 2002 and January 2006 and living in the study area of Rotterdam were invited to participate. In total, 9778 women (response 61%) were enrolled in the study of which 8880 women during pregnancy and another 898 at the birth of their child. The information required for this study was collected in the questionnaire completed during mid-pregnancy (around approximately 30 weeks of gestation) by 6830 women (77% of enrolment) and information on pregnancy complications was obtained from medical records. For this study we selected women who were prenatally enrolled, with paid employment before or during pregnancy, with no history of pre-existing hypertension (blood pressure ≥140/90 mmHg before 20 weeks of gestation)[4] and with a spontaneously conceived singleton liveborn pregnancy (n = 4465). Spontaneously conceived refered to pregnancies achieved without assisted reproductive techniques, such as ovulation induction or in vitro fertilisation. For each mother, we included the first pregnancy within the Generation R cohort in our study, since some women participated with more than one child in the study. The flowchart of the study population is depicted in Figure 1. The study was approved by the Medical Ethics Committee at Erasmus University Medical Centre Rotterdam, The Netherlands (MEC 198.782/2001/31). Written consent was obtained from all participants.

Hypertensive disorders during pregnancy

Information on pregnancy complications was obtained from medical records. Women who delivered in hospital and who had chronic hypertension or were reported to have experienced PIH (>140/90 mmHg) or hypertension related complications (preeclampsia, proteinuria, eclampsia, and/or HELLP syndrome) were selected from hospital registraties. Their individual medical records were subsequently studied by qualified medical doctors, who defined pregnancy induced hypertension, preeclampsia and eclampsia according to the criteria of the International Society for the Study of Hypertension in Pregnancy (ISSHP) and according to those of the College of Obstetricians and Gynaecologists (ACOG). Blood pressure measurements were performed during pregnancy in early, mid and late pregnancy until gestational week 32-34.

FIGURE 1. Flowchart of the study population

Details of these procedures have been described elsewhere.[28] Briefly, the following criteria were used to identify woman with PIH: development of systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24-hour urine collection containing at least 300 mg of protein) were used to identify woman with preeclampsia.[29]

Occupation and working conditions

The mid-pregnancy questionnaire contained questions about work status, occupation, and working conditions and focussed on the periconception and pregnancy period. Work status, based on a single question on the current economic status with seven categories (paid labour, self-employed, unemployed, disabled, homemaker, student, or other), was used to select women with paid employment, consisting of women within the first two categories. This question was followed by questions whether the mother had worked before conception in this current occupation, and the starting and (optional) stop date of this current occupation. We selected women who started working before conception and women who started working somewhere during the first trimester of pregnancy. Further questions on job title, type of business, name of employer, and activities in the job were used to classify jobs into the Dutch Classification of Occupations[30] and subsequently link these codes to a Job-Exposure-Matrix (JEM) for chemical exposure. This new JEM was developed according to a general strategy, comprising of a literature search to identify chemicals, information gathering on occupations at risk, and literature on occupational settings in which the selected chemicals were encountered and exposure measurements were performed. This reference material served as a starting point for the expert assessment. Three experts were asked to estimate exposures based on their knowledge of tasks and working environment in various occupations. Finally, exposure probability scores were added based on the judgement of three experts. For various chemicals, subjects experience a certain level of exposure through diet, environment or widely used consumer products. The JEM exposure score refers to the probability of occupational exposure, which is assumed to exceed the background level in the general population. The exposure probability scores were assigned by means of consensus discussions in which the original scores were taken into account where possible, but no prior individual assessments were performed. The JEM comprises ten categories of chemicals, namely polycyclic aromatic hydrocarbons (PAH), polychlorinated organic compounds, pesticides, phthalates, organic solvents, bisphenol A, alkylphenolic compounds, flame retardants, metals, and miscellaneous agents.[31] For 353 job titles, probability scores were classified into three levels: 'unlikely'(0), 'possible'(1), and 'probable'(2). For this study we collate the last two categories into one category indicating possible exposure to chemicals. The category 'any chemicals' combines all women exposed to one of the groups of chemicals defined in the JEM. Different country specific JEMs have been used in various studies, and the JEM is a valuable tool for exposure assessment in epidemiological

studies on the health risks of chemical exposure.[31-35] The questions on physically demanding work were based on the Dutch Musculoskeletal Questionnaire[36] and concerned questions on manually handling loads of 25 kg or more, long periods of standing, long periods of walking, long periods of driving, night shifts, and working hours. These questions were part of the mid-pregnancy questionnaire distributed around approximately 30 weeks of gestation. A four-point scale was used with ratings 'seldom or never', 'occasionally', 'often', and 'very often' during a regular workday.[36] We reclassified long periods of standing and walking into three categories, namely 'seldom or never', 'occasionally', and 'often/very often'. We reclassified long periods of driving, manual handling of load of 25 kg or more and night shifts into two categories, namely 'seldom or never' and 'occasionally/often/very often'. The number of weekly working hours of the mothers with paid employment was assessed by means of an open question, 'How many hours per week do you work?'. Working hours were categorised into '1-24', '25-39', and '40 or more hours a week'.[37]

Potential confounders

The following variables were considered as possible confounders in the association between physically demanding work, exposure to chemicals and hypertensive disorders during pregnancy: maternal age, pre-pregnancy weight, height, educational level, ethnicity, parity, smoking, alcohol use, and folic acid supplement use. Information about maternal age, educational level, ethnicity, parity, and folic acid supplement use was obtained by questionnaire at enrolment in the study. Maternal smoking habits and alcohol use were assessed in three prenatal questionnaires in each trimester and classified into three categories, namely no smoking or alcohol use, smoking or alcohol use until pregnancy was known, and smoking or alcohol use during pregnancy.[38] Maternal height was measured at intake in the study. Body mass index (BMI) was calculated as weight divided by squared height.

Statistical analysis

We used bivariable and multivariable logistic regression analyses to study the association between maternal characteristics, occupational risk factors and hypertensive disorders during pregnancy. We reclassified age, a continuous variable, into four categories for ease of interpretation. Individual characteristics significantly associated with PIH or preeclampsia were considered for the multivariable analyses. The final model consisted of the following confounders: maternal age, educational level (both included by default), ethnicity, parity, and BMI. Two sensitivity analyses were carried out, first we stratified the analyses for Dutch versus non-Dutch women, secondly we assessed whether women who quitted working before 34 weeks of gestation because of pregnancy complaints had a higher risk of PIH or preeclampsia. This information on the gestational week women stopped working was available for 3537 women (68.6%). Missing values in confounders were handled by multiple imputations (fully conditional specification, Markov Chain Monte Carlo method) by generating five independent datasets for all

analyses, using SPSS version 17.0 for windows. Variables included in the imputation procedure (these variables were both imputed and used as predictors of missing data) were: maternal age, educational level, ethnicity, parity, pre-pregnancy weight, height at intake, smoking during pregnancy, alcohol use during pregnancy, folic acid supplement use, foetal gender, and gestational age at birth. Table 1 presents the proportion of missing values for each variable that was imputed. All multivariable analyses were performed with the multiple imputation datasets, and pooled estimates were calculated across these five independent datasets. The maximal allowed threshold for imputations was set on a maximum of missing values of 30%.[39] All logistic regression analyses were performed using Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

The characteristics of the study population are shown in Table 1. Age at enrolment ranged from 17.0 to 46.0 years with a mean age of 31.1 years. The largest ethnic group was from Dutch origin (63.7%), and Surinamese and Dutch Antillean women (8.1%), and Turkish and Moroccan women (7.2%) were less represented. The prevalence of PIH and preeclampsia in our study population was 1.8% (79 cases) and 1.3% (60 cases), respectively. The occupational characteristics are presented in Table 2.

The bivariable analysis in Table 3 shows associations between individual characteristics and hypertensive disorders during pregnancy. Multiparous women were at lower risk for both PIH and preeclampsia. Compared to Dutch mothers, women from other ethnic minorities showed a lower risk on PIH (OR 0.47; 95%CI 0.23-0.94). For preeclampsia, we observed that Surinamese and Dutch Antillean women showed a significantly higher risk of preeclampsia (OR 2.23; 95%CI 1.08-4.57). Overweight and obese mothers had increased risks of PIH and preeclampsia. Smoking and alcohol consumption were not associated with PIH or preeclampsia.

Table 4 shows the bivariable and multivariable logistic regression analyses between occupational risk factors and hypertensive disorders during pregnancy. There were no consistent associations between physically demanding work and chemical exposure with hypertensive disorders during pregnancy. For almost all risk factors related to physically demanding work we could not find a clear exposure-response relation, and women 'often' exposed to a certain occupational risk factor were not consistently at higher risk for hypertensive disorders during pregnancy compared to women who were 'occasionally' exposed. When we restricted the analysis to primigravidous women (63% of the study population), the effect estimates remained very similar to the presented effect estimates in Table 4 (data not shown). Furthermore, there were no differences in effect estimates between Dutch and non-Dutch women. Women quitting their job before 34 weeks of gestation were significantly at higher risk of PIH (OR 1.81; 95%CI 1.04-3.14) and at higher risk of preeclampsia (OR 1.92; 95%CI 0.96-3.84), although not statistically significant (data not shown).

TABLE 1. Baseline characteristics of pregnant women participating in a birth cohort study, the Generation R Study (n = 4465)

Maternal characteristics		**Results**
Age at intake (years)		31.09 (4.5)
Weight before pregnancy (kg)		69.35 (12.44)
Height measured at intake (cm)		168.78 (7.14)
Educational level	Low	611 (13.7%)
	Mid-low	1273 (28.5%)
	Mid-high	1076 (24.1%)
	High	1365 (30.6%)
	Missing	140 (3.1%)
Ethnicity	Netherlands	2845 (63.7%)
	Surinam and Dutch Antilles	360 (8.1%)
	Morocco and Turkey	322 (7.2%)
	Other	847 (19.0%)
	Missing	91 (2.0%)
Parity	Nulliparous	2826 (63.3%)
	Multiparous	1520 (34.0%)
	Missing	119 (2.7%)
Body Mass Index (BMI)	<25 kg/m^2	2724 (61.0%)
	25-30 kg/m^2	1016 (22.8%)
	>30 kg/m^2	380 (8.5%)
	Missing	345 (7.7%)
Smoking	No	2899 (64.9%)
	Yes, until pregnancy was known	332 (7.4%)
	Yes, during pregnancy	519 (13.8%)
	Missing	715 (16.0%)
Alcohol	No	1459 (32.7%)
	Yes, until pregnancy was known	559 (12.5%)
	Yes, during pregnancy	1757 (39.4%)
	Missing	690 (15.5%)
Folic acid use	No	555 (12.4%)
	Yes, post conception start	1097 (24.6%)
	Yes, preconception start	1662 (37.2%)
	Missing	1151 (25.8%)
Hypertensive disorders during pregnancy	Preeclampsia	60 (1.3%)
	Pregnancy induced hypertension	79 (1.8%)

Values are means (SD) for normally distributed continuous variables or medians (minimum–maximum) for skewed distributed continuous variables, and absolute numbers (percentages) for categorical variables.

TABLE 2. Occupational characteristics of pregnant women participating in a birth cohort study, the Generation R Study (n = 4465)

Occupational characteristics		Results
Long periods of standing	No	2329 (52.2%)
	Occasionally	881 (19.7%)
	Often/very often	840 (18.8%)
	Missing	415 (9.3%)
Long periods of walking	No	2036 (45.6%)
	Occasionally	1399 (31.3%)
	Often/very often	634 (14.2%)
	Missing	396 (8.9%)
Long period of driving	No	3499 (78.4%)
	Occasionally/often/very often	572 (12.8%)
	Missing	394 (8.8%)
Lifting or carrying weights >25kg	No	3815 (85.4%)
	Occasionally/often/very often	267 (6.0%)
	Missing	383 (8.6%)
Night shift (each month)	No	3892 (87.2%)
	Occasionally/often/very often	188 (4.2%)
	Missing	385 (8.6%)
Working hours	<25 hours per week	1163 (26.0%)
	25-39 hours per week	2112 (47.3%)
	>40 hours per week	1040 (23.3%)
	Missing	150 (3.4%)
Exposure to chemicals (JEM)	PAH	55 (1.2%)
	Pesticides	22 (0.5%)
	Phthalates	65 (1.5%)
	Organic solvents	213 (4.8%)
	Alkylphenolic compounds	150 (3.4%)
	Metals	51 (1.1%)
	Any chemicals	297 (6.7%)

Values are means (SD) for normally distributed continuous variables or medians (minimum–maximum) for skewed distributed continuous variables, and absolute numbers (percentages) for categorical variables.

TABLE 3. Associations in a birth cohort study among pregnant women on maternal individual characteristics and hypertensive disorders during pregnancy

Maternal characteristics		**PIH** OR (95% CI)	**Preeclampsia** OR (95% CI)
Age before intake	<25 years	1.00	1.00
	25-29 years	2.07 (0.79-5.46)	2.39 (0.82-6.95)
	30-35 years	1.70 (0.66-4.37)	1.67 (0.58-4.81)
	>35 years	2.19 (0.80-6.01)	1.20 (0.35-4.11)
Educational level	Low	0.82 (0.39-1.72)	1.91 (0.90-4.07)
	Mid-low	1.05 (0.61-1.82)	1.77 (0.92-3.40)
	Mid-high	0.82 (0.44-1.51)	0.53 (0.19-1.44)
	High	1.00	1.00
Ethnicity	Netherlands	1.00	1.00
	Surinam and Dutch Antilles	0.71 (0.31-1.67)	2.23 (1.08-4.57)*
	Morocco and Turkey	0.26 (0.06-1.05)	1.29 (0.50-3.34)
	Other	0.47 (0.23-0.94)*	1.10 (0.55-2.20)
Parity	Nulliparous	1.00	1.00
	Multiparous	0.55 (0.33-0.94)*	0.21 (0.09-0.49)**
Body Mass Index (BMI)	<25 kg/m^2	1.00	1.00
	25-30 kg/m^2	2.86 (1.66-4.93)**	2.19 (1.26-3.80)*
	>30 kg/m^2	7.96 (4.57-13.88)**	2.20 (1.00-4.84)
Smoking	No	1.00	1.00
	Yes, until pregnancy was known	0.92 (0.39-2.17)	0.37 (0.09-1.54)
	Yes, during pregnancy	0.99 (0.48-2.01)	0.81 (0.36-1.80)
Alcohol	No	1.00	1.00
	Yes, until pregnancy was known	0.98 (0.50-1.92)	0.99 (0.45-2.16)
	Yes, during pregnancy	0.85 (0.51-1.42)	0.89 (0.51-1.57)
Folic acid use	No	1.00	1.00
	Yes, post conception start	1.61 (0.74-3.51)	1.16 (0.52-2.55)
	Yes, preconception start	1.44 (0.69-3.01)	1.00 (0.48-2.11)

*p<0.05 **p<0.01

TABLE 4. Associations in a birth cohort study among pregnant women on physically demanding work, chemical exposure and hypertensive disorders during pregnancy

Occupational characteristics		PIH		Preeclampsia	
		OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)
Long periods of standing	No	1.00	1.00	1.00	1.00
	Occasionally	1.02 (0.58-1.80)	1.05 (0.59-1.88)	1.12 (0.60-2.11)	1.01 (0.52-1.93)
	Often/very often	1.00 (0.56-1.78)	1.16 (0.62-2.15)	1.00 (0.51-1.94)	0.87 (0.43-1.78)
Long periods of walking	No	1.00	1.00	1.00	1.00
	Occasionally	1.55 (0.95-2.55)	1.68 (1.00-2.81)*	0.82 (0.46-1.47)	0.74 (0.41-1.35)
	Often/very often	1.45 (0.77-2.74)	1.74 (0.87-3.47)	1.00 (0.49-2.05)	0.77 (0.37-1.67)
Long periods of driving (>4 hours)	No	1.00	1.00	1.00	1.00
	Occasionally/often/very often	0.75 (0.42-1.34)	1.30 (0.87-1.47)	0.93 (0.45-1.89)	0.77 (0.37-1.60)
Lifting or carrying weights > 25 kg	No	1.00	1.00	1.00	1.00
	Occasionally/often/very often	0.84 (0.36-1.95)	0.92 (0.39-2.18)	0.98 (0.35-2.72)	1.07 (0.38-3.01)
Night shifts (each month)	No	1.00	1.00	1.00	1.00
	Occasionally/often/very often	0.57 (0.24-1.32)	0.59 (0.25-1.42)	0.89 (0.28-2.88)	0.86 (0.26-2.80)
Working hours	<25 hours per week	1.00	1.00	1.00	1.00
	25-40 hours per week	0.91 (0.53-1.54)	0.67 (0.38-1.20)	1.14 (0.57-2.26)	0.81 (0.40-1.66)
	> 40 hours per week	0.71 (0.32-1.38)	0.43 (0.20-0.90)*	1.74 (0.85-3.59)	1.04 (0.48-2.26)
Exposure to chemicals (JEM)	PAH	2.99 (0.91-9.77)	2.64 (0.74-9.35)	1.28 (0.17-9.43)	0.89 (0.12-6.75)
	Pesticides	-	-	3.14 (0.42-23.73)	3.15 (0.38-25.94)
	Phthalates	-	-	1.05 (0.14-7.72)	0.82 (0.11-6.16)
	Organic solvents	0.72 (0.22-2.29)	0.94 (0.29-3.09)	0.96 (0.30-3.08)	0.92 (0.28-3.04)
	Alkylphenolic compounds	1.04 (0.32-3.34)	1.56 (0.46-5.29)	0.91 (0.22-3.75)	0.81 (0.19-3.45)
	Metals	-	-	2.72 (0.65-11.43)	2.21 (0.50-9.67)
	Any chemicals	1.05 (0.45-2.44)	1.22 (0.51-2.94)	1.17 (0.46-2.93)	1.04 (0.40-2.68)

* p-value < 0.05.

Effect estimates were adjusted for maternal age, educational level, parity, ethnicity and BMI.

DISCUSSION

In this large population-based prospective birth cohort study we were not able to find a consistent association between physically demanding work and exposure to chemicals with hypertensive disorders during pregnancy. These results suggest that there is no effect of occupational risk factors on the occurrence of hypertensive disorders during pregnancy. The main limitation of this study is the limited number of women with PIH or preeclampsia and the low prevalence of exposure to chemicals.

The findings in our study corroborates with the conclusions from a recent review that the available evidence on the presence of an association between physically demanding work and PIH or preeclampsia was not sufficient to propose restrictions in activities during pregnancy.[17] However, another review reported a clear association between physically demanding work during pregnancy and preeclampsia.[16] We hypothesised that the contradictory findings in the scientific literature may be partly due to heterogeneity in the definition of PIH and preeclampsia across studies, and also in the definitions of physically demanding work, which makes comparisons difficult. In our study, we used strict criteria to assess hypertensive complications during pregnancy. Medical records were checked and the diagnosis was made by qualified medical doctors. The low prevalence of these disorders in our study population can be explained by the strict criteria for diagnosis. Furthermore, blood pressure measurements in our study were performed until gestational week 32-34. Thereafter, medical records were checked for the occurrence of PIH and preeclampsia, this might have led to a lower incidence of PIH, since this disease may have no clear pattern of symptoms, and often, hospital admission is not required. Another explanation for the low prevalence may be the selection of women with paid employment, since these women generally have better pregnancy outcomes than women without paid employment.[32,37] In our analyses, we choose women with paid employment, to avoid 'health worker bias'.[37] Furthermore, women with pregnancy complications may quit their job earlier during pregnancy than healthy women, and technically these women would be on sick leave. The sensitivity analyses on women who reported stopping working before 34 weeks because of pregnancy complaints showed that these women were at higher risk of PIH and preeclampsia. However, quitting before 34 weeks of gestation was not associated with physically demanding work nor exposure to chemicals and, thus, will not have influenced the reported associations. Since women from ethnic minorities may also have higher risks of adverse pregnancy outcomes, we carried out stratified analyses, however, effect estimates were comparable, indicating no differences.

For occupational exposure to chemicals and hypertensive disorders during pregnancy, the evidence is scarce and contradictory. Irwin et al. found no relation between occupational exposure to chemicals and hypertensive disorders during pregnancy,[18] whereas Eskenazi and Saldana reported associations between solvents and pesticides with PIH and preeclampsia.[25,26] In our study, exposure to pesticides showed an increased risk of preeclampsia (OR 3.15; 95%CI

0.38-25.94). However, this was not statistically significant, probably due to the low number of women exposed to pesticides (n = 23). We must conclude that the prevalence of occupational exposure to chemicals in the general population is very low, and, thus, the proportion of PIH and preeclampsia attributable to occupational exposure will be low.

One of the suggested mechanisms through which physically demanding work could lead to hypertensive disorders during pregnancy is an increased uteroplacental vascular resistance which follows physical exertion.[40] Physically demanding work may cause an increase in catecholamine levels, which may lead to a decreased uterine blood flow and therefore may induce PIH and preeclampsia.[12] It has also been suggested that part of the excess catecholamine release is due to an overactive sympatic nerve system.[41] For exposure to chemicals, the underlying mechanisms are largely unclear.

Exposure assessment is an important issue in this study. For assessment of maternal exposure to chemicals we used a recently updated Job-Exposure-Matrix (JEM).[31-35] This approach assured that exposures status was blinded to participants and researchers, both aspects which avoid information bias. The characterisation of exposure in the JEM must be interpreted as exposure probabilities. However, if misclassification occurred, this is most likely non-differential misclassification, leading to underestimation of the effect estimates. A major drawback of JEMs is that they do not account for variability in tasks and working environments within job titles. Furthermore, the JEM does not contain specific chemicals, but only contains broad groups of chemicals, and the mechanisms of action can vary between specific chemicals in a group. However, from the task description, it may become clear that some subjects within a specific job title, for example subjects who have odd jobs around a farm (feeding animals) are less likely to be exposed to pesticides. Background exposure to various chemicals through diet and environment may occur. However, it is unlikely that background exposure is associated with occupational exposure, thus, background exposure will not confound the relation between occupational chemical exposure and hypertensive disorders during pregnancy. Furthermore, it is expected that levels of exposure to chemicals within occupations are generally much higher than general exposure through diet and environment. Since we did not assess background exposure, it may have contributed to unexplained variance in the outcome hypertensive disorders during pregnancy. In our study we classified physically demanding work in three or two relevant levels of exposure, however, this approach does not quantify the exposure into hours of physically demanding work performed per day, and therefore is at best a semi-quantitative measure. Furthermore, this study did not take into account other sources of physically demanding activities outside employment, such as exercise, housework, and volunteer work. However, it is unlikely that these activities are strongly related to physically demanding work risk factors, but they may lead, in some extent, to residual confounding.

In order to assess whether there was any overlap between the occupational risk factors in this study, we calculated kappa values for all exposure categories. Kappa values ranged from

0.00-0.18, indicating that there was almost no overlap between physically demanding work and exposure to chemicals in the workplace.

The strength of this study is the population-based approach with recruitment during the prenatal period and the availability of a large number of potential risk factors. Within the Generation R cohort, Bakker et al. showed that smoking during the first trimester is associated with maternal cardiovascular adaptations during pregnancy.[42] Gaillard et al. showed that there is a strong relation between obesity and PIH and preeclampsia.[43] Thus, in our analysis we could adjust for these well-established risk factors. A limitation of this study is the selective participation whereby mothers from ethnic minorities, those with lower socio-economic status, and mothers or children with medical complications, were less represented in the study population than expected in the population of Rotterdam.[44] This non-response will lead to biased effect estimates if the association between physically demanding work, chemical exposure and hypertensive disorders during pregnancy differs between participants and non-responders. However, this seems unlikely since biased estimates in large cohort studies mainly arise from loss to follow up rather than from non-response at baseline.[45] Selective participation may have influenced the prevalence of exposure to physically demanding work and chemicals, but bias is unlikely since physically demanding work and exposure to chemicals was assessed independently from and prior to the hypertensive disorders during pregnancy. Although we were able to control for a large number of potential confounders, residual confounding cannot be ruled out completely. Recall bias in this study is unlikely, since the information obtained was not biased by the outcome since the questionnaire was completed in mid-pregnancy. In this study we used multiple imputation for missing values in covariates. This reduces bias due to non-random missing in the covariates.

In summary, this large population-based birth cohort study suggests that physically demanding work and exposure to chemicals did not influence the occurrence of hypertensive disorders during pregnancy. However, the very low prevalence of PIH and preeclampsia in our study, combined with the low prevalence of occupational risk factors, may have resulted in too little discriminatory power to detect such associations.

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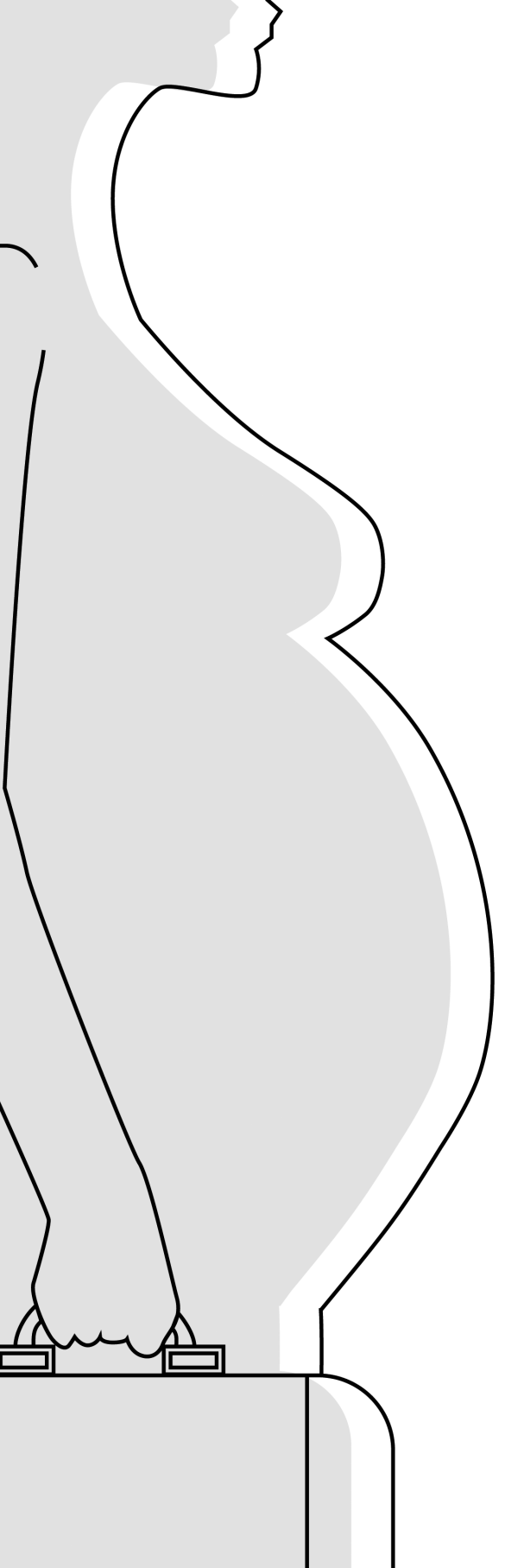

Chapter 3.2

Physically demanding work, foetal growth, and adverse birth outcomes

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ABSTRACT

Objectives: Work-related risk factors, such as long work hours, and physically demanding work have been suggested to adversely influence pregnancy outcome. The authors aimed to examine associations between various aspects of physically demanding work with foetal growth in different trimesters during pregnancy and the risks of adverse birth outcomes.

Methods: Associations between physically demanding work and foetal growth were studied in 4680 pregnant women participating in a population-based prospective cohort study from early pregnancy onwards in the Netherlands (2002-2006). Mothers who filled out a questionnaire during mid-pregnancy (response 77% of enrolment), were included if they conducted paid employment and had a spontaneously conceived singleton live born pregnancy. Questions on physical work load were obtained from the Dutch Musculoskeletal Questionnaire and concerned questions on lifting, long periods of standing or walking, night shifts, and working hours. Foetal growth characteristics were repeatedly measured by ultrasound and were used in combination with measurements at birth.

Results: There were no consistent significant associations between physically demanding work nor working hours in relation to small-for-gestational-age, low birth weight or preterm delivery. Women exposed to long periods of standing had lower growth rates for foetal head circumference, resulting in a reduction of approximately 1 cm (3%) of the average head circumference at birth. Compared with women working <25 hours per week, women working 25-39 hours per week, and > 40 hours per week had lower growth rates for both foetal weight and head circumference, resulting in a difference of approximately 1 cm in head circumference at birth and a difference of 148-198 grams in birth weight.

Conclusion: Long periods of standing and long working hours per week during pregnancy seem to negatively influence intrauterine growth.

INTRODUCTION

Developmental diseases, such as structural birth defects, functional alterations, growth restrictions, and preterm delivery account for more than 25% of infant mortality and morbidity.[1,2] Environmental exposures and lifestyle behaviours act at different stages of foetal development and may result in adverse birth outcomes, such as preterm birth, low birth weight, small-for-gestational-age, certain congenital defects, and foetal death.[3-5] Although women in paid employment seem to have better pregnancy outcomes than those without paid jobs,[6-8] certain work-related risk factors, such as exposure to chemicals,[9] long working hours,[7,10] high physical work load, prolonged standing,[11] and psychological job strain[12,13] have been suggested to adversely influence pregnancy outcome.

Two reviews have summarised the literature on physical workload and adverse pregnancy outcomes. Mozurkewich et al. performed a meta-analyses on 29 studies, and concluded that physically demanding work may significantly increase risks of preterm delivery (pooled OR 1.22; 95%CI 1.16-1.29), small-for-gestational-age (pooled OR 1.37; 95%CI 1.30-1.44), and hypertension or preeclampsia (pooled OR 1.60; 95%CI 1.30-1.96).[14] A review by Bonzini et al. on 49 studies described the relation between five common occupational exposures (prolonged working hours, shift work, lifting, standing and heavy physical work load) and three major adverse outcomes, namely preterm delivery, low birth weight, and preeclampsia/gestational hypertension.[15] Due to the small effects, low population attributable fractions, and conflicting results, mandatory restrictions were not justified.

Variations in study findings may be due to differences in exposure assessment, definitions of physical work load and components of indices that were used to score physical workload and timing during pregnancy, the same activity may carry different risks if it occurred late in pregnancy compared with only a few weeks after conception. Despite the substantial body of evidence on physically demanding work and birth outcomes, it is still unclear how occupational activities of pregnant women should be managed. Furthermore, studies on physically demanding work have primarily focussed on adverse birth outcomes, which are important from an obstetric point of view, but are rather crude measures of foetal growth. To gain more insight in how physically demanding work influences birth outcomes, studies on foetal growth characteristics during pregnancy are needed in order to identify critical periods in which exposure is deleterious for foetal growth and development.

The aims of this study were to examine associations between various aspects of physically demanding work with foetal growth in different trimesters during pregnancy and the risks of adverse birth outcomes.

MATERIALS AND METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from foetal life until young adulthood in Rotterdam, the Netherlands.[16,17] Briefly, all pregnant women who had an expected delivery date between April 2002 and January 2006 and lived in the study area of Rotterdam were invited to participate. In total, 9778 pregnant women (response 61%) were enrolled in the study of which 8880 women were enrolled during pregnancy and another 898 at birth of their child. Extensive assessments were carried out in each trimester, including physical examinations, questionnaires, interviews, and biological samples. The study was approved by the Medical Ethics Committee at Erasmus University Medical Centre Rotterdam, The Netherlands (MEC 198.782/2001/31).

The occupational information required for this study was collected in the questionnaire completed during mid-pregnancy (send out at 30 weeks of gestation), which was filled out by 6830 women (77% of enrolment). The mean gestational age for completing the questionnaire was 30.8 weeks (standard deviation 2.4 weeks). For this study we selected women who were prenatally enrolled, with paid employment at the time of the questionnaire (5154 women), and we excluded women with twin pregnancies (76 women), with pregnancies of non-spontaneous origin (84 women), and with foetal death (4 women). For each woman we included the first pregnancy within the Generation R cohort in our study, excluding women that participated more than once (310 women). The population for analysis consisted of 4680 women, the flowchart of the study population in depicted in Supplement 1.

Foetal ultrasounds

For this study we used the ultrasound measures of foetal head circumference, and estimated foetal weight, since these measures are essential characteristics to describe foetal growth. In our research facility, we measured foetal head circumference (HC), abdominal circumference (AC), and femur length (FL) to the nearest millimetre using standardised ultrasound procedures in the second (median 20.5, minimum-maximum 18.0-25.0 weeks) and third (median 30.4, minimum-maximum 25.8-37.0 weeks) trimester. Since use of the last menstrual period for pregnancy dating has several limitations,[18] and a large number of women in our study population did not know the exact date of their last menstrual period (76%), we used crown-rump length for pregnancy dating until a gestational age of 12 weeks (2308 women) and biparietal diameter for pregnancy dating thereafter (2372 women) in all women.[19,20] First trimester measurements (3459 women) were primarily used to establish gestational age and therefore not included in the growth analyses. Estimated foetal weight (EFW) was calculated using the formula by Hadlock et al.[21] The intraclass correlation coefficient of foetal growth measurements was 0.95, tested on 21 subjects.[22]

Verburg et al. showed that foetal growth reference curves for foetal weight and foetal head circumference during pregnancy typically have a parabolic pattern. Based on these reference curves, standard deviation (SD) scores for all growth characteristics were constructed,[18] reflecting the commonly used z-scores for child growth as proposed by the World Health Organisation.[23] The SD score indicates the relative position of the foetus on the observed distribution, for example a SD score of one for foetal head circumference indicates for that particular child his HC measurement is larger than approximately 84% of all children. This approach enables linear analyses of the foetal growth characteristics since the reference curve is a curve with a mean SD score of 0.

Birth outcomes

Information about gender at birth, gestational age, weight, length, and head circumference at birth was obtained from medical records and hospital registries. Low birth weight was defined as birth weight <2500 gram. Small-for-gestational-age at birth was defined as a gestational age adjusted birth weight below the 5th percentile in the whole study cohort (n = 8880) (<-1.71 standard deviation), and preterm birth was defined as a gestational age at <37 weeks at birth.

Occupation and working conditions

The mid-pregnancy questionnaire (send out at 30 weeks of gestation) contained questions about work status, occupation, and working conditions and focussed on the periconception and pregnancy period. The question on current work status, with seven categories (paid labour, self-employed, unemployed, disabled, homemaker, student or other), was used to select women with paid employment. The question on starting date of the current occupation provided information if women started working before pregnancy or somewhere during the first trimester of pregnancy. For the current study we both selected women with paid employment who started before pregnancy, and women who started working during the first trimester.

The number of weekly working hours of the mothers with paid employment was assessed by means of an open question, 'How many hours per week do you work?'. Working hours were categorised into '1-24', '25-39', and '40 or more hours a week'.[7] The questions on physical workload were obtained from the Dutch Musculoskeletal Questionnaire and concerned questions on manually handling loads of 25 kg or more, long periods of walking, long periods of standing, and night shifts. A four-point scale was used with ratings 'seldom or never', 'occasionally', 'often', and 'very often' during a regular workday. These factors were all considered as separate variables in the analyses, since multicollinearity was not present (Spearman's correlation coefficient ρ = -0.14 to 0.23) except for long periods of standing and walking (ρ = 0.56). We reclassified long periods of walking and standing, manual handling of load of 25 kg or more and night shifts into three categories, namely 'seldom or never' (reference group), 'occasionally', and 'often/very often'.[24,25] In a postnatal questionnaire we collected information in which pregnancy week women had stopped working, and whether this was due to pregnancy complaints.

Potential confounders

The following variables were considered as possible confounders: maternal age, pre-pregnancy weight, height, educational level, ethnicity, parity, smoking, alcohol use, and folic acid supplement use. Information about maternal age, educational level, ethnicity, parity, and folic acid supplement use was obtained by questionnaire at enrolment in the study. Maternal smoking habits and alcohol use were assessed on the basis of three questionnaires in each trimester and classified as no, until pregnancy was known, or during pregnancy.[26,27] Maternal height was measured at intake in the study.

Statistical analyses

The associations between occupational risk factors and the risk of preterm delivery, small-for-gestational-age, and low birth weight were analysed with multiple logistic regression analyses. In all analyses, the reference group consisted of women who were not exposed to that particular physical risk factor. Second, cross-sectional analyses were performed using linear regression analysis to demonstrate the influence of physically demanding work on head circumference, abdominal circumference and estimated foetal weight in the second and third trimester of pregnancy, respectively. Third, occupational risk factors associated with birth outcomes were selected for the longitudinal analyses of head circumference, and weight (second- and third trimester estimated foetal weight and birth weight) using unbalanced repeated measurement analysis, which enables optimal use of the available data, taking into account correlations within subjects and assessing both time dependant and independent associations. In these linear longitudinal models, we used standard deviation (SD) scores as parameter of foetal growth (dependent variable). The final model can be written as follows (e.g., for foetal weight): SD score of foetal weight = $ß_0 + ß_1$ x gawks + $ß_2$ x exposuregroup + $ß_3$ x gawks x exposuregroup (gawks = gestational age in weeks). In this model, $ß_0$ reflects the intercept and $ß_2$ x exposuregroup tests the difference in intercept between exposed and non-exposed group. The coefficient $ß_3$ reflects the slope (interaction of exposure with gestational age), and tests whether the groups of exposed and non-exposed grow at the same rate over time. The latter coefficient is the main interest for this article, since this beta represents the average decline or increase in SD for foetal weight per gestational week for exposed women versus non-exposed women. The regression models were adjusted for lifestyle and socioeconomic confounders used in previous studies on maternal occupational exposure[6] and known determinants of foetal growth: maternal age, educational level, ethnicity, parity, pre-pregnancy weight, height at intake, smoking during pregnancy, alcohol use during pregnancy, folic acid supplement use, and foetal gender.

Missing values in confounders were handled by multiple imputations (fully conditional specification, Markov Chain Monte Carlo method) by generating five independent datasets for all analyses, using SPSS version 17.0 for windows. Variables included in the imputation procedure (these variables were both imputed and used as predictors of missing data) were: maternal age, educational level, ethnicity, parity, pre-pregnancy weight, height at intake, smoking

TABLE 1. Baseline characteristics of the study population (n = 4680)

Variables		Results
Age at intake (yr)		31.08 (4.56)
Weight before pregnancy (kg)		64.00 (34.00-145.00)
Height measured at intake (cm)		168.80 (7.12)
Educational level	Low	653 (14.0%)
	Mid-low	1333 (28.5%)
	Mid-high	1129 (24.1%)
	High	1419 (30.3%)
	Missing	146 (3.1%)
Ethnicity	Netherlands	2993 (64.0%)
	Surinam and Dutch Antilles	380 (8.1%)
	Morocco and Turkey	328 (7.0%)
	Other	885 (18.9%)
	Missing	94 (2.0%)
Parity	Nulliparous	2992 (63.9%)
	Multiparous	1565 (33.4%)
	Missing	123 (2.6%)
Smoking	Yes, during pregnancy	546 (11.7%)
	Yes, until pregnancy was known	355 (7.6%)
	No	3031 (64.8%)
	Missing	748 (16.0%)
Alcohol	Yes, during pregnancy	1846 (39.4%)
	Yes, until pregnancy was known	587 (12.5%)
	No	1524 (32.6%)
	Missing	723 (15.4%)
Folic acid use	No	580 (12.4%)
	Yes, post conception start	1163 (24.9%)
	Yes, preconception start	1735 (37.1%)
	Missing	1202 (25.7%)
Maternal occupational characteristics	Long periods of standing, occasionally	920 (19.7%)
	Long periods of standing, often	883 (18.9%)
	Long periods of walking, occasionally	1467 (31.3%)
	Long periods of walking, often	665 (14.2%)
	Lifting > 25kg, occasionally	217 (4.6%)
	Lifting > 25kg, often	70 (1.5%)
	Night shifts, occasionally	137 (2.9%)
	Night shifts, often	60 (1.3%)
	Work hours 1-24 hours/week	1193 (25.5%)
	Work hours 25-39 hours/week	2222 (47.5%)
	Work hours > 40 hours/week	1087 (23.2%)

TABLE 1. Baseline characteristics of the study population (n = 4680) (**continued**)

Variables		Results
Growth outcomes	Second trimester ultrasonography	4197 (89.7%)
	Third trimester ultrasonography	4294 (91.8%)
Birth outcomes	Gestational age at birth (wk)	40.14 (22.71-43.43)
	Birth weight (grams)	3449.81 (549.28)
	Male	2365 (50.5%)
	Head circumference at birth (mm)	33.89 (1.65)
	Length at birth (mm)	50.33 (2.38)
Low birth weight (<2500 g)	Yes	203 (4.3%)
	No	4674 (99.9%)
	Missing	6 (0.1%)
Small for gestational age (<-1.7 SD)	Yes	201 (4.3%)
	No	4463 (95.7%)
	Missing	16 (0.3%)
Preterm delivery (<37 weeks of gestation)	Yes	231 (4.9%)
	No	4449 (95.1%)

Values are means (standard deviation) for normal distributed continuous variables or medians (minimum-maximum) for skewed distributed continuous variables, and absolute numbers (percentages) for categorical variables.

during pregnancy, alcohol use during pregnancy, folic acid supplement use, foetal gender, and gestational age at birth. Table 1 presents the proportion of missing values for each variable that was imputed. All multivariable analyses were performed with the multiple imputation datasets, and pooled estimates were calculated across these five independent datasets. The maximal allowed threshold for imputations was set on a maximum of missings values of 30%. However, missing values for parameters of physically demanding work were not imputed and, thus, the analysis on each exposure of interest was based on slightly different number of subjects due to some missing values.

In total, three sensitivity analyses were performed, the first to evaluate whether women who started working before conception differed from women who started working during the first trimester, the second to analyse whether women with a certain last menstrual period and regular cycle differed from women whose pregnancy was dated by means of an ultrasound, and the third to study the influence of the subgroup of women who stopped working before 34 weeks of gestation because of pregnancy complaints. Results from the logistic regression analyses on birth outcomes were used to estimate population attributable fractions (PAFs), expressing the proportion of the adverse health outcomes in the general population that is attributable to the risk factors of interest.[28] The repeated measurement analyses were conducted with the Proc Mixed module of the Statistical Analysis System (version 9.2; SAS Institute Inc, Cary NC).

RESULTS

Table 1 shows the baseline characteristics of the study population. In total, 38.6% of the women were exposed to long periods of standing at work, 45.5% to long periods of walking at work, and 6.1% to heavy lifting at work. About 4.2% of the women regularly worked night shifts. Part-time jobs were common among women, since 47.5% worked 25-39 hours per week, 25.5% worked less than 25 hours per week, and 23.2% worked more than 40 hours per week.

Table 2 shows the associations between maternal occupational exposure to physically demanding work and adverse birth outcomes. There were no consistent associations between physically demanding work, long working hours and adverse birth outcomes. Furthermore, there was no clear dose-response relation, and women often exposed to a certain occupational risk factor were not consistently at higher risk for adverse birth outcomes. In these multivariable models with adverse birth outcomes, the following confounders significantly influenced the outcome (in descending order of magnitude): maternal age, pre-pregnancy weight, height at intake, parity, ethnicity, smoking and folic acid use. Joint effects of several physically demanding work risk factors and working hours were investigated, however, we did not found any statistically significant joint effect on adverse birth outcomes (data not shown).

Table 3 shows the cross-sectional analyses between long periods of standing, lifting > 25 kg, and working hours with head circumference, abdominal circumference and estimated foetal weight during the second (~20 weeks of gestation) and third (~30 weeks of gestation) trimester in pregnancy. After adjustment for potential confounders, no associations of physically demanding work or working hours with foetal growth characteristics during the second trimester were found. In the third trimester of pregnancy, after adjustments for potential confounders, long periods of standing was significantly associated with a decreased foetal head circumference. For working hours we observed effects on abdominal circumference and estimated foetal weight, however, after adjustment for potential confounders, these effects did not remain statistically significant.

Figures 1 and 2 show the association between long periods of standing and working hours on longitudinally measured growth (foetal weight and foetal head circumference). Long periods of standing at work were associated with slower growth rates in head circumference (-0.32 SD and -0.33 SD at birth), which corresponds to approximately 1 cm difference (3%) compared to the average head circumference of 33.9 cm at birth. Women working >25 hours per week showed reduced foetal growth rates in both domains of foetal growth, namely foetal weight and head circumference. In these models, educational level and ethnicity significantly influenced foetal growth, but did not influence the relation between physically demanding work and foetal growth, resulting in comparable effect estimates.

In total, 4177 (89.3%) women filled out the question concerning the starting date of their current occupation, 4068 women (97.4%) started working before conception, whereas 109 (2.6%) women started working somewhere during their first trimester of pregnancy. In the

TABLE 2. Associations between maternal occupational exposure to physically demanding work during pregnancy with the risk of adverse birth outcomes in the Generation R Study

Physically demanding work	N	SGA (n=201)		Preterm birth (n=231)		Low birth weight (n=203)	
		OR (95%CI) unadjusted	OR (95%CI) adjusted[a]	OR (95%CI) unadjusted	OR (95%CI) adjusted[a]	OR (95%CI) unadjusted	OR (95%CI) adjusted[b]
Long periods of standing							
no	2435	Reference	Reference	Reference	Reference	Reference	Reference
occasionally	918	1.49 (1.06-2.11)*	1.34 (0.94-1.92)	0.83 (0.57-1.20)	0.78 (0.53-1.15)	1.20 (0.84-1.73)	1.65 (1.02-2.64)*
often	881	1.04 (0.70-1.54)	0.95 (0.63-1.45)	1.03 (0.72-1.46)	0.95 (0.65-1.40)	1.00 (0.68-1.47)	1.02 (0.60-1.73)
Long periods of walking							
no	2129	Reference	Reference	Reference	Reference	Reference	Reference
occasionally	1461	1.19 (0.86-1.65)	1.09 (0.78-1.53)	1.12 (0.83-1.53)	1.07 (0.78-1.46)	1.16 (0.84-1.60)	1.08 (0.70-1.67)
often	664	1.40 (0.94-2.08)	1.22 (0.79-1.89)	0.96 (0.63-1.46)	0.85 (0.54-1.33)	0.96 (0.61-1.50)	0.86 (0.46-1.60)
Lifting > 25kg							
no	3981	Reference	Reference	Reference	Reference	Reference	Reference
occasionally	217	2.11 (1.27-3.50)*	2.41 (1.43-4.08)*	1.26 (0.71-2.25)	1.26 (0.70-2.26)	1.21 (0.65-2.25)	1.31 (0.60-2.85)
often	70	1.79 (0.71-4.51)	1.85 (0.70-4.88)	0.58 (0.14-2.39)	0.55 (0.13-2.28)	1.01 (0.32-3.25)	1.86 (0.44-7.77)
Nightshifts							
no	4069	Reference	Reference	Reference	Reference	Reference	Reference
occasionally	137	1.74 (0.90-3.37)	1.69 (0.86-3.33)	0.90 (0.39-2.07)	0.87 (0.38-2.01)	1.04 (0.45-2.39)	1.78 (0.64-4.96)
often	60	0.76 (0.19-3.15)	0.73 (0.17-3.08)	1.41 (0.51-3.92)	1.29 (0.46-3.65)	1.62 (0.58-4.52)	1.23 (0.28-5.41)
Work hours							
1-24 hours/week	1191	Reference	Reference	Reference	Reference	Reference	Reference
25-39 hours/week	2214	1.38 (0.94-2.02)	1.19 (0.79-1.77)	1.44 (1.01-2.05)*	1.29 (0.89-1.87)	1.51 (1.03-2.21)*	1.25 (0.75-2.09)
>40 hours/week	1084	1.65 (1.09-2.52)*	1.29 (0.81-2.05)	1.58 (1.06-2.35)*	1.32 (0.85-2.03)	1.73 (1.14-2.64)*	1.20 (0.66-2.18)

* p-value < 0.05

[a] Adjusted for maternal age, height at intake, weight before pregnancy, educational level, ethnicity, parity, smoking, alcohol use, folic acid supplement use, self-perceived health, and foetal gender.

[b] Adjusted for maternal age, height at intake, weight before pregnancy, educational level, ethnicity, parity, smoking, alcohol use, folic acid supplement use, gestational age at birth, self-perceived health, and foetal gender.

TABLE 3. Associations between maternal occupational exposure to physically demanding work during pregnancy and foetal growth measured by ultrasound, second and third trimester analyses

Physically demanding work	N	Head circumference (mm) 20 weeks		N	Head circumference (mm) 30 weeks	
		Crude*	Adjusted†		Crude*	Adjusted†
Long period of standing	4065			4152		
no	2344	Reference	Reference	2385	Reference	Reference
occasionally	876	-0.11 [-0.47; 0.45]	0.16 [-0.30; 0.61]	906	**-1.17 [-1.84; -0.49]**	**-0.80 [-1.46; -0.14]**
often	845	-0.24 [-0.71; 0.23]	0.05 [-0.44; 0.54]	861	**-1.36 [-2.06; -0.67]**	**-0.72 [-1.43; -0.01]**
Lifting > 25kg	4099			4184		
no	3822	Reference	Reference	3900	Reference	Reference
occasionally	209	0.70 [-0.12; 1.52]	0.66 [-0.15; 1.47]	214	0.01 [-1.20; 1.23]	-0.13 [-1.30; 1.04]
often	68	-0.58 [-2.03; 0.87]	-0.30 [-1.73; 1.13]	70	-1.61 [-3.75; 0.53]	-0.96 [-3.02; 1.10]
Work hours	4011			4094		
1-24 hours/wk	1020	Reference	Reference	1051	Reference	Reference
25-39 hours/wk	2002	-0.03 [-0.47; 0.41]	-0.11 [-0.56; 0.34]	2027	-0.32 [-0.97; 0.33]	-0.32 [-0.97; 0.33]
>40 hours/wk	989	0.03 [-0.48; 0.55]	-0.13 [-0.69; 0.44]	1016	-0.03 [-0.79; 0.73]	-0.07 [-0.88; 0.75]

TABLE 3. Associations between maternal occupational exposure to physically demanding work during pregnancy and foetal growth measured by ultrasound, second and third trimester analyses **(continued)**

		Abdominal circumference (mm) 20 weeks			**Abdominal circumference (mm) 30 weeks**	
		Crude*	**Adjusted†**		**Crude***	**Adjusted†**
Long period of standing	4080			4179		
no	2354	Reference	Reference	2402	Reference	Reference
occasionally	879	-0.57 [-1.18; 0.03]	-0.37 [-0.98; 0.24]	907	-0.95 [-1.91; 0.01]	-0.59 [-1.55; 0.37]
often	847	-0.31 [-0.93; 0.31]	-0.03 [-0.69; 0.63]	870	-0.71 [-1.70; 0.28]	0.02 [-1.02; 1.06]
Lifting > 25kg	4114			4212		
no	3837	Reference	Reference	3928	Reference	Reference
occasionally	209	0.37 [-0.72; 1.45]	0.27 [-0.82; 1.35]	214	-0.13 [-1.85; 1.60]	-0.31 [-2.02; 1.40]
often	68	-0.16 [-2.08; 1.76]	0.02 [-1.89; 1.93]	70	-0.01 [-3.06; 3.04]	0.23 [-2.77; 3.23]
Work hours	4027			4120		
1-24 hours/wk	1024	Reference	Reference	1057	Reference	Reference
25-39 hours/wk	2011	-0.31 [-0.89; 0.26]	-0.26 [-0.86; 0.35]	2038	**-1.08 [-2.00; -0.16]**	-0.57 [-1.52; 0.38]
>40 hours/wk	992	-0.67 [-1.36; 0.01]	-0.57 [-1.33; 0.18]	1025	**-1.34 [-2.42; -0.26]**	-0.51 [-1.70; 0.68]

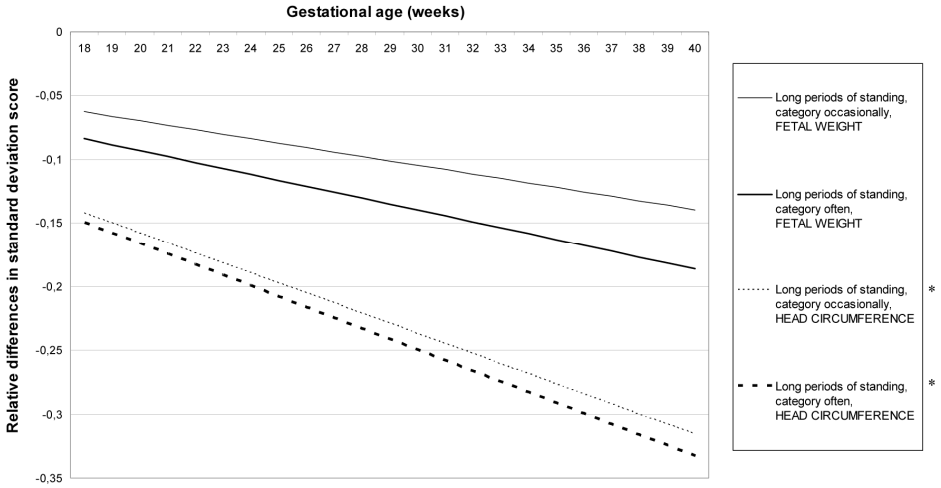
TABLE 3. Associations between maternal occupational exposure to physically demanding work during pregnancy and foetal growth measured by ultrasound, second and third trimester analyses **(continued)**

		Estimated foetal weight (grams) 20 weeks			**Estimated foetal weight (grams) 30 weeks**	
		Crude*	**Adjusted†**		**Crude***	**Adjusted†**
Long period of standing	4063			4172		
no	2343	Reference	Reference	2398	Reference	Reference
occasionally	876	-0.65 [-3.86; 2.57]	-0.41 [-3.68; 2.85]	906	-13.06 [-26.70; 0.63]	-7.56 [-21.23; 6.12]
often	844	0.21 [-3.10; 3.52]	0.04 [-3.49; 3.56]	868	-6.42 [-20.52; 7.67]	2.47 [-12.38; 17.31]
Lifting > 25kg	4096			4205		
no	3821	Reference	Reference	3923	Reference	Reference
occasionally	207	5.41 [-0.38; 11.21]	4.10 [-1.71; 9.91]	212	6.24 [-18.52; 31.01]	1.50 [-22.96; 25.96]
often	68	2.01 [-8.20; 12.22]	1.23 [-8.99; 11.45]	70	9.74 [-33.76; 53.24]	12.32 [-30.52; 55.17]
Work hours	4009			4113		
1-24 hours/wk	1019	Reference	Reference	1055	Reference	Reference
25-39 hours/wk	2002	0.05 [-3.03; 3.13]	1.65 [-1.59; 4.89]	2034	**-15.35 [-28.47; -2.24]**	-5.69 [-19.23; 7.84]
>40 hours/wk	988	-2.78 [-6.41; 0.85]	0.02 [-4.02; 4.06]	1024	**-19.52 [-34.93; -4.10]**	-3.84 [-20.72; 13.05]

* adjusted for gestational age at ultrasound

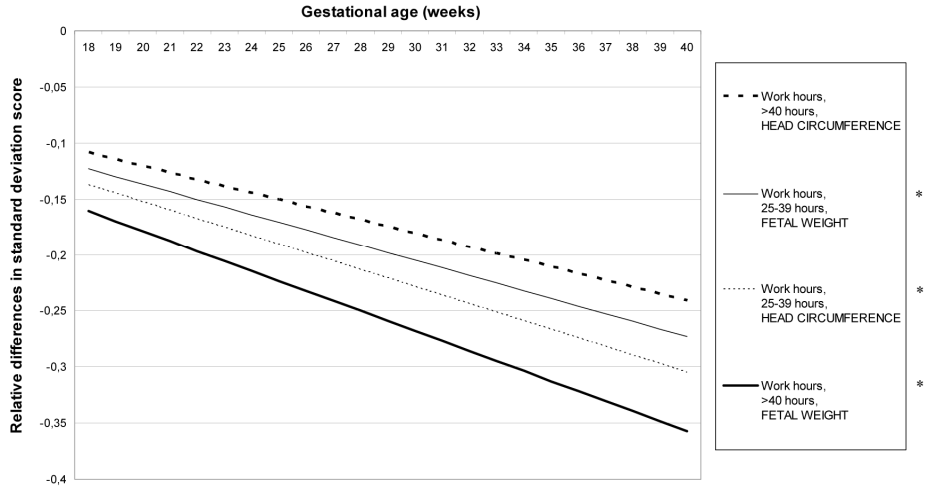
† adjusted for gestational age at ultrasound, maternal age, height at intake, weight before pregnancy, educational level, ethnicity, parity, smoking, alcohol use, folic acid supplement use, self-perceived health, and foetal gender.

FIGURE 1. Relative differences in SD scores for women occupationally exposed to long periods of standing at work compared to non-exposed women, for foetal weight and foetal head circumference.



Values are based on repeated linear regression models and reflect the difference in SD score of foetal weight (12467 measurements), and foetal head circumference (10540 measurements), in the offspring of mothers exposed to long periods of standing at work compared to the offspring of non-exposed mothers. The reference value is a SD score of 0. * = p-vaue < 0.05. Models were adjusted for: maternal age, height at intake, weight before pregnancy, educational level, ethnicity, parity, smoking, alcohol use, folic acid supplement use, foetal gender, and self-perceived health.

FIGURE 2. Relative differences in SD scores for women who work 25-39 or >40 hours per week compared to women who work <25 hours per week, for foetal weight, and foetal head circumference



Values are based on repeated linear regression models and reflect the difference in SD score of foetal weight (12611 measurements), and foetal head circumference (10411 measurements), in the offspring of mothers who work 25-39 or >40 hours per week compared to the offspring of mothers who work <25 hours per week. The reference value is a SD score of 0. * = p-value < 0.05. Models were adjusted for: maternal age, height at intake, weight before pregnancy, educational level, ethnicity, parity, smoking, alcohol use, folic acid supplement use, foetal gender, and self-perceived health.

sensitivity analyses starting work before or during conception and having a regular menstrual cycle or not did not change the results. Furthermore, women who stopped working earlier than planned (before 34 weeks of gestation) often had medical reasons (71.4%) and these women had a higher risk of preterm delivery and low birth weight. When excluding these women from the analysis, duration of work during pregnancy was not associated with foetal growth and we found no negative effect of working till 34-36 weeks of pregnancy on any of the birth outcomes in this study population. Stopping working before 34 weeks of gestation was not associated with physically demanding work or working hours.

DISCUSSION

This population-based prospective cohort study suggests that long periods of standing at work, and working > 25 hours per week were associated with lower foetal growth rates for foetal weight and head circumference in pregnancy. These findings were not reflected in adverse birth outcomes. Additional cross-sectional analyses showed that the differences are demonstrable from the third trimester onwards.

Several mechanisms have been suggested to explain the possible adverse influence of physically demanding work during pregnancy on the foetus. Heavy physical work is thought to reduce the blood flow to the uterus and placenta, thereby reducing the availability of oxygen and nutrients for the foetus.[29,30] Furthermore, lifting and trunk bending may increase intra abdominal pressure, which in turn may lead to preterm delivery, especially in the last trimester when space in the abdominal cavity is maximally constrained.[10] Also an increased release of catecholemines, through mediation of the sympatic nerve system, has been hypothesised to play a role.[31] Occupational risk factors, such as working in a specific occupation,[8,32] shift work,[33,34] job stress,[12,13,35] standing, lifting,[36] and work hours[37-39] have been related to adverse birth outcomes. Two reviews have suggested an influence of physically demanding work on pregnancy outcomes.[14,15] In addition to previous studies, which looked at adverse birth outcomes, we have looked at foetal growth measured in the second and third trimester of pregnancy. Although birth outcomes are important from an obstetric perspective, they are rather crude measures of foetal growth during pregnancy. We could not demonstrate an effect of working hours > 25 hours per week on adverse birth outcomes, however, effects on foetal growth rates during pregnancy could be demonstrated, suggesting that the latter analyses are more sensitive for picking up more subtle differences in foetal weight and head circumference. The population attributable fractions (PAFs) for the occupational risk factors in this study were small, for SGA with the highest contribution of lifting > 25 kg, PAF 4.2%, for preterm delivery with the highest contribution of working > 40 hours per week, PAF 1.5%, and for low birth weight with the highest contribution of lifting > 25 kg per week, PAF 3.6%. In this community based study physically demanding work had little influence on the prevalence of adverse birth

outcomes, but in specific occupations with a high prevalence of physically demanding work this contribution could be higher.

Long working hours were associated with impaired foetal weight, resulting in a decrease in SD at birth varying between -0.27 and -0.36 SD at birth. This corresponds to approximately 150 to 200 gram difference in birth weight. This effect seems of similar magnitude than the effects of other well-known lifestyle factors, such as smoking and caffeine intake with reported reductions of -0.3 SD and -0.1 to -0.3 SD.[27,40] However, we must note that the population attributable fractions of specific categories of physically demanding work were very low, and the effects on foetal growth were subtle since these effects were not reflected in adverse birth outcomes. The results of the current study hampers sound advice for pregnant women exposed to these risk factors.

Women working as nurse, child care giver or saleswoman most often reported lifting heavy loads (together accounting for 44.6% of all working women). For standing, several occupations were reported, most notably saleswoman, working with toddlers, schoolteachers and administrative employees (21.5%). Nightshifts were most frequently reported by stewardesses, physicians, and nurses (60.3%).

In this study we used ultrasound measurements for pregnancy dating, which seems superior to dating based on the last menstrual period.[18] A disadvantage is that growth variations in early pregnancy are assumed to be zero, impairing analyses on first trimester growth. The repeated measurements based on gestational age adjusted SD scores, comparable to standardised z-scores, enables us to identify pathological smallness in stead of constitutional smallness. The advantage of SD scores as relative measure of difference is that the SD scores can be used in linear regression models, whereas absolute differences in foetal growth were highly skewed since growth curves during pregnancy have a typical parabolic shape that must be described by fractional polynomials instead of normal distributions.

The strength of this study is the population-based approach with recruitment during the prenatal period and the availability of a large number of potential confounders. A limitation of this study is lower selective participation among mothers from ethnic minorities and with lower socio-economic status.[41] The non-response would lead to biased effect estimates if the association between physically demanding work and foetal development would be different between those included and those not included in the analyses. However, this seems unlikely since biased estimates in large cohort studies mainly arise from loss to follow up rather than from non-response at baseline.[42] Information on psychosocial stress or general fatigue, which could correlate with working hours and foetal growth, was not available in this study. Furthermore, this study did not take into account other sources of physically demanding activities outside employment, such as exercise, housework, and volunteer work. However, it is unlikely that these activities are strongly related to physically demanding work risk factors, but they may lead, in some extent, to residual confounding. Women working in physically demanding jobs could have a more unhealthy lifestyle that was not fully adjusted for in the analysis by including smoking and alcohol use as confounders. Education is an important determinant of health behaviour, but

adjustment for educational level did not affect the relation between physically demanding work and birth outcomes or foetal growth. This suggests that life style related risk factors most likely do not bias the relation between long working hours and foetal growth.

A limitation of this study is the semi-quantative nature of the exposure information in four self-reported categories. This did not allow us to investigate duration of standing and walking per week or frequency of lifting heavy weights. Recall bias is unlikely, since the information obtained was not biased by the outcome since the questionnaire was completed in mid-pregnancy. In this study we used multiple imputations for missing values in covariates. This reduces selection bias due to non-random missing in the covariates.

In the current study we selected women with paid employment around week 30 of pregnancy and this might have resulted in a more healthy and affluent study population since these women generally have better pregnancy outcomes than women without paid employment. Women in paid employment might have stopped working earlier during pregnancy due to pregnancy complaints, and technically these women would be on sick leave. The sensitivity analyses on women who reported stopping working before 34 weeks because of pregnancy complaints showed that these women were at higher risk of preterm delivery and low birth weight. However, this was not associated with physically demanding work and, thus, will not have influenced the reported associations. When excluding these women from the analysis, duration of work during pregnancy was not associated with foetal growth. We were unable to find a clear negative effect of working till 34-36 weeks of pregnancy on any of the birth outcomes in this study population. When we corrected the longitudinal models for the duration of work during pregnancy (thus pregnancy week when women stopped working) it did not change the effect estimates, suggesting that the relation between physically demanding work and foetal growth is independent of work duration.

In the study, we found that physically demanding work during pregnancy was associated with lower foetal growth rates. We believe that optimising the work environment is important since participation of women in the reproductive age in the work force continues to increase. Preventive measures reducing certain occupational conditions, such as shift work, night hours, standing, lifting, and noise, have proven to reduce the risks of adverse birth outcomes.[33,43] In the current study we were unable to pinpoint the effects of physically demanding work in specific trimesters or of cumulative exposure over pregnancy, since occupational activities were only measured once during pregnancy. We were able to demonstrate differences in foetal growth during the third trimester, and we hypothesised that differences might already originate earlier during pregnancy, but were too small to be noticed. Preventive measures therefore may be most beneficial when focussing on the weeks before the third trimester. However, this study does not present concrete information on the required reduction in duration and level of work demands, which hampers sound advice. The results of this study need to be confirmed by future research.

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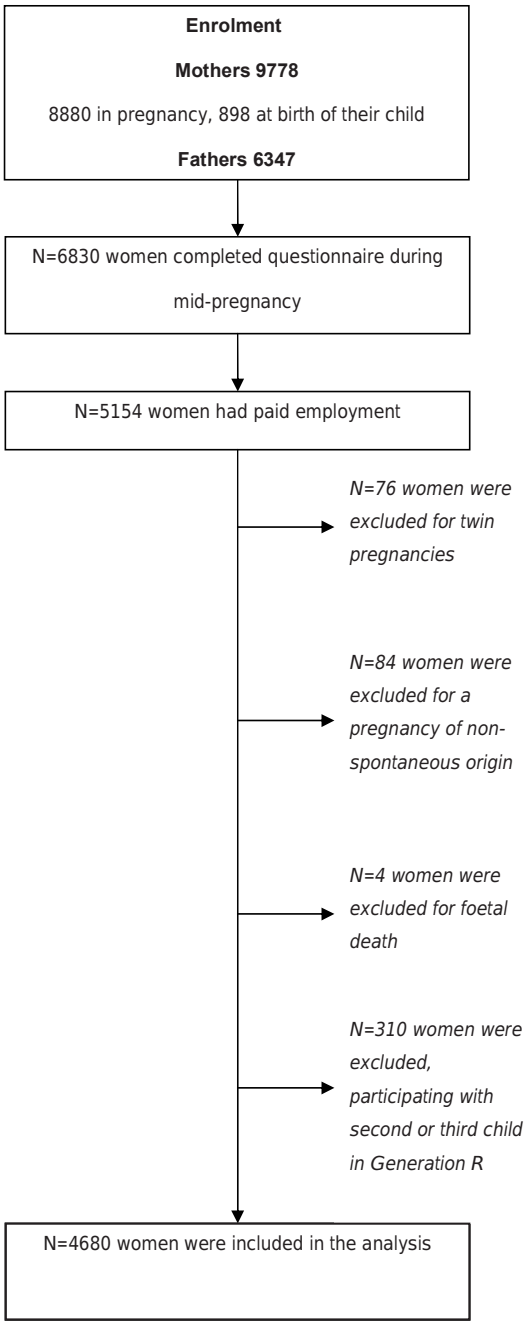
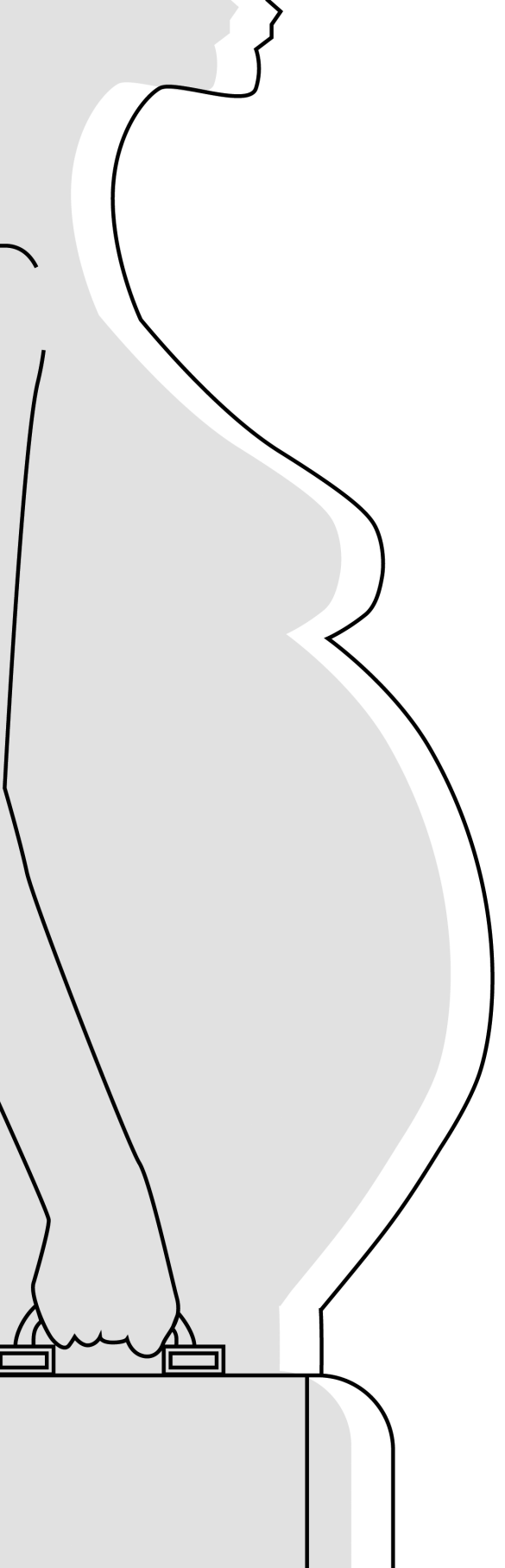
SUPPLEMENT 1. Flowchart of the study population



PART 4

MILD ANALGESICS AND REPRODUCTIVE DISORDERS



Chapter 4.1

Mild analgesic use during pregnancy and reproductive disorders

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ABSTRACT

Background: Recently, over-the-counter mild analgesic use during pregnancy has been suggested to influence the risk of reproductive disorders in the offspring. We examined the influence of maternal exposure to mild analgesics during pregnancy on the occurrence of cryptorchidism and hypospadia in their offspring.

Methods: Associations between maternal exposure to mild analgesics during pregnancy and cryptorchidism or hypospadia in the offspring were studied in 3184 women participating in a large population-based prospective birth cohort study from early pregnancy onwards in the Netherlands (2002-2006), the Generation R Study. Cryptorchidism and hypospadia were identified during routine screening assessments performed in child health care centres by trained physicians. The use of mild analgesics was assessed in three prenatal questionnaires in pregnancy, resulting in four periods of use, namely, periconception period, first 14 weeks of gestation, 14-22 weeks of gestation, and 20-32 weeks of gestation. Logistic regression analyses were used to study the associations between maternal exposure to mild analgesics and cryptorchidism and hypospadia.

Results: The cumulative prevalence over 30 months of follow up was 2.1% for cryptorchidism and 0.7% for hypospadia. Use of mild analgesics in the second period of pregnancy (14-22 weeks) increased the risk of congenital cryptorchidism (adjusted OR 2.12; 95%CI 1.17 to 3.83), primarily due to the use of acetaminophen (paracetamol) (adjusted OR 1.89; 95%CI 1.01 to 3.51). Among mothers of cryptorchid sons, 33.8% reported (23 of 68) the use of mild analgesics during pregnancy, compared with 31.8% (7 of 22) of mothers with a boy with hypospadia, and 29.9% (926 of 3094) of mothers with healthy boys.

Conclusion: Our results suggest that intrauterine exposure to mild analgesics, primarily paracetamol, during the period in pregnancy when male sexual differentiation takes place, increases the risk of cryptorchidism.

INTRODUCTION

Congenital anomalies are a significant cause of stillbirth and infant mortality and are also important contributors to childhood morbidity.[1] Although cryptorchidism (undescended testis) is one of the most common abnormalities in newborn boys worldwide, the aetiology in boys without chromosomal abnormalities is largely unknown.[2,3] Reproductive disorders, including cryptorchidism, hypospadia, and poor semen quality are hypothesised to constitute a testicular dysgenesis syndrome, in which environmental and genetic factors play a role.[4,5]

Use of medication, such as diethylstilbestrol (DES) and valproic acid therapy, during pregnancy increases the risk of congenital malformations, including cryptorchidism and hypospadia.[6-8] Recently, some evidence was presented that over-the-counter mild analgesic use may also increase the risk of cryptorchidism in the offspring.[9] In the Netherlands, approximately 40% of the population uses over-the-counter self medication such as acetaminophen (paracetamol) and non-steroidal anti-inflammatory drugs (NSAIDS), which are also used by pregnant women.[10,11]

Experimental rat models have shown that normal androgen action during a critical male programming window (gestational day 15.5 to 17.5) is crucial for the programming of the testis decent.[12] Factors that diminish androgen action during that time may have detrimental consequences for male sexual differentiation.[4] Exposure of pregnant rats to phthalate esters during gestational days 15-17 resulted in hypospadia, cryptorchidism, testicular injury, and nipple retention in male offspring, and this was attributed to reductions in testosterone synthesis.[13] A recent study by Kristensen et al. showed that paracetamol, even at low plasma concentrations such as 1 μM, is a potent inhibitor of testosterone production, reducing anogenital distance and testosterone production in rats.[9] Furthermore, COX inhibitors, such as acetaminophen, ibuprofen, and acetylsalicyclic acid have shown endocrine disrupting properties in rainbow trout, affecting steroid hormone synthesis.[14]

These experimental observations have found echos in human observational studies. As early as 1996, Berkowitz and Lapinski reported that the use of analgesics during pregnancy was a risk factor for cryptorchidism.[15] A recent study by Jensen et al. among 47400 live born children in the Danish National Birth Cohort showed that exposure to acetaminophen in both the first and second trimester increased the risk of cryptorchidism.[16] Kristensen et al. were able to substantiate these observations among a different cohort of Danish pregnant women, and observed that combined use of acetaminophen with other analgesics further increased the risk of cryptorchidism.[9] However, similar associations were not observed among Finnish mothers and their boys, possibly because this disorder is comparatively rare in Finland.[9] Further research is therefore urgently needed to corroborate or refute these findings.

The aim of this study was to investigate whether the use of mild analgesics during pregnancy by mothers was associated with an increased occurrence of cryptorchidism and hypospadia in their offspring. We conducted this study within the Generation R Study, a large prospective

birth cohort study from early pregnancy onwards examining determinants of growth, development and health from foetal life until young adulthood.[17]

MATERIALS AND METHODS

Study design

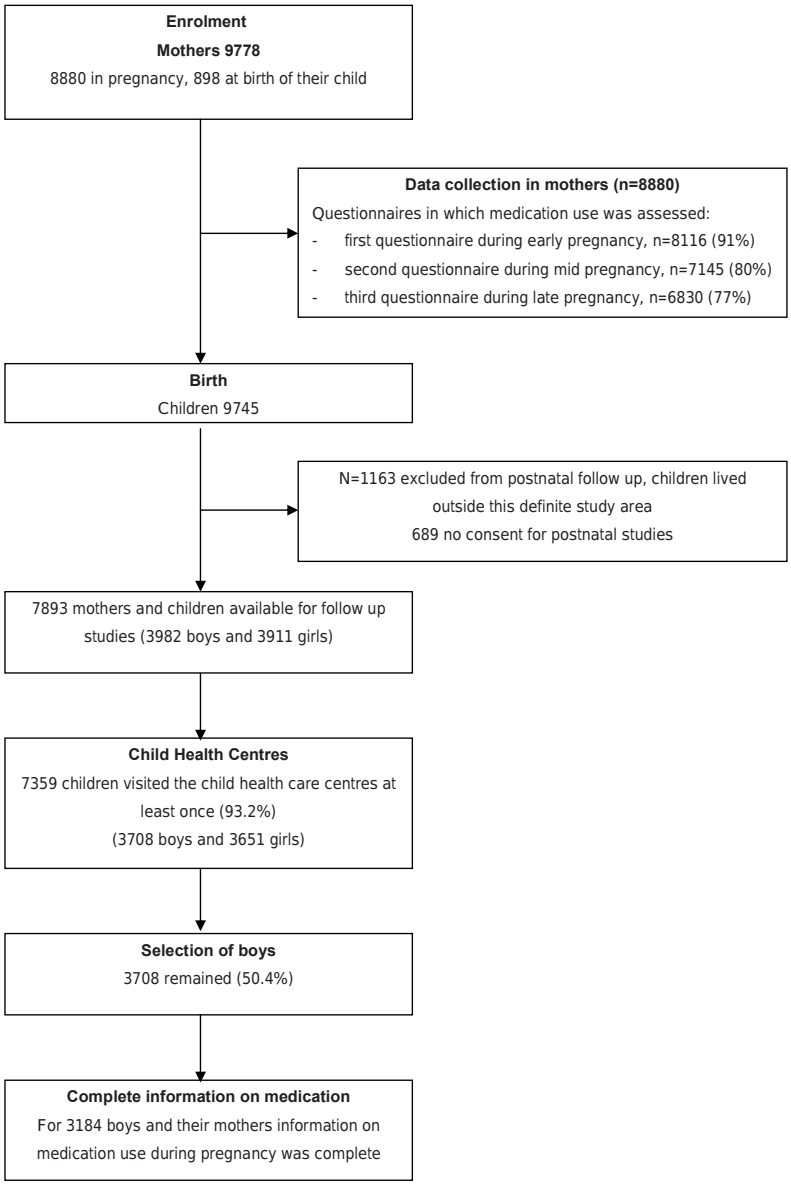
The Generation R Study is a population-based prospective cohort study on growth, development, and health from early foetal life until young adulthood in Rotterdam, the Netherlands. The study design has been described in detail previously.[17] Briefly, all pregnant women who had an expected delivery date between April 2002 and January 2006 and lived in the study area of Rotterdam were invited to participate. In total, 9778 pregnant women (response 61%) were enrolled in the study of which 8880 women were enrolled during pregnancy and another 898 at birth of their child. Extensive assessments were carried out during early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18-25 weeks), and late pregnancy (gestational age > 25 weeks), including physical examinations, questionnaires, interviews, and biological samples. The study was approved by the Medical Ethics Committee at Erasmus University Medical Centre Rotterdam, The Netherlands (MEC 198.782/2001/31). Written informed consent was obtained from all participants. The information required for this study was collected in three prenatal questionnaires completed during the first trimester (8116 women, 91% enrolment), the second trimester (7145 women, 80% enrolment), and the third trimester (6830 women, 77% enrolment). In total, 5177 partners completed a questionnaire during mid-pregnancy (82% of enrolment). The analyses were restricted to boys, and the presence of cryptorchidism and hypospadias was ascertained during ten visits of children to the child health care centres (0-48 months). The flowchart of the study population is depicted in Figure 1.

Medication use

The three self-administered questionnaires assessed medication use during pregnancy and were send out by post at gestational week 12, 20, and 30 with an average lag period to response of approximately two weeks. The first questionnaire (sent out at 12 weeks of gestation) contained the following question 'Did you use any medication during the past six months?', whereby we explicitly asked for medication prescribed by a physician and medication bought over-the-counter such as analgesics. This question was followed by a scheme in which mothers had to fill out the name of the medication, reason for use, age at start using, use during pregnancy, and if they stopped using when the pregnancy was known.

The second questionnaire (sent out at 20 weeks of gestation) contained the following question 'Did you use any medication during the past two months?' and the third questionnaire (sent out at 30 weeks of gestation) a similar question but focussing on the past three months.

FIGURE 1. Flowchart of the selected study population



We defined four time periods, namely, use during the periconception period (use before and during the first trimester of pregnancy), use during the first period (first 14 weeks of gestation), use during the second period (14-22 weeks of gestation) and use during the third period (20-32 weeks of gestation). Women enrolled during early pregnancy (85% of the study population) completed the first questionnaire around 14 weeks of gestation (mean). Women who reported

medication use before pregnancy and use during the first weeks of pregnancy until the pregnancy was known or thereafter were classified as periconception users, and women who used during the first weeks of pregnancy until the pregnancy was known or thereafter (strict selection from the group of periconception users) were classified as first period users (0-14 weeks of gestation). Women who were included after 20 weeks (15% of the study population), respectively 30 weeks (5% of the study population), were included in the second and/or third period analyses. Summarising, for 2724 (85.6%) women information on three time periods was available, for 443 (13.9%) women information on two time periods was available, and for 17 (0.5%) women information on one time period was available. Use of mild analgesics in these four time periods was classified into use of paracetamol as over-the-counter medication, and use of all other painkillers, including NSAIDS and aspirin, on prescription or over-the-counter.

Reproductive disorders

The presence of cryptorchidism and hypospadia was assessed during routine screening assessments performed in child health care centres. Child health care centres are notified of live births within two days after registration in the municipal birth register. Child health care centres invite all parents to participate in a national preventive child healthcare programme, free of charge. A total of ten visits were planned at different ages, namely 0-6 months (five visits, 6591 children, 84% enrolment), 6-12 months (one visit, 6414 children, 81% enrolment), 12-18 months (two visits, 6088 children, 77% enrolment), 18-24 months (one visit, 4478 children, 57% enrolment), and 24-36 months (one visit, 5335 children, 68% enrolment). All visits included physical examinations, performed by trained physicians, including manipulation of the testes and inspection of the genitalia.[2]

Testes deviating from the normal distal scrotal position were gently but firmly manipulated with warm hands, along the normal pathway of the descent, to their most distal position.[18] Boys were diagnosed as cryptorchid if one or both testes were non-palpable, or when they could not be manipulated to a stable position in the scrotum. Retractile testis can be manipulated to a stable scrotal position were not considered cryptorchid, whereas cases of retentio testis manipulated to a scrotal position that returned to their abnormal position after release of pressure were classified as cryptorchid. The physicians reported whether the tests were performed, and reported the position of the testis after manipulation, as non-palpable, inguinal, ectopic, high scrotal, stable scrotal, or non-assessable due to the presence of hydrocele. Hypospadias were also diagnosed and classified in the children. Trained physicians assessed whether hypospadia was present and which type it was (glandular of a more severe type), as described earlier by Pierik et al.[19]

When cryptorchidism or hypospadia was present at one of the ten visits to the child health care centres, children were classified as a prevalent case. This resulted in a cumulative period prevalence of cryptorchidism and hypospadia over 30 months of follow up.

Potential confounders

For mothers, information on age, weight, education, country of origin, parity, underlying diseases, folic acid supplement use, and general health was collected from the first questionnaire available. Smoking habits and alcohol use (no/until pregnancy was known/ and after pregnancy was known), infectious diseases during pregnancy (yes/no), fever during pregnancy (yes/no), and use of co-medication (yes/no) were collected from three prenatal questionnaires. The body mass index (BMI in kg/m^2) was calculated by weight divided by squared height. The occurrence of 'underlying disease' was defined as the presence of any disease from a structured list of 23 questions on specific diseases in the first questionnaire. General health was reclassified from five categories varying from excellent to poor into two categories comparing moderate and poor health to good to excellent health. Gestational age at birth and birth weight were obtained from medical records and hospital registries. Information on paternal characteristics, such as paternal age, BMI, education, country of origin, underlying diseases, smoking before pregnancy, alcohol use before pregnancy, occupational exposure to endocrine disruptors and pesticides, medication use before pregnancy, and family history of congenital cryptorchidism, was collected from a mid-pregnancy questionnaire especially designed for partners of participating women.

Statistical analyses

Statistical analyses were performed using SPSS v17.0 (Chicago, IL, USA). Differences between categorical variables were tested with the Fisher's exact test, and differences between continuous variables were tested with one-way ANOVA. We used logistic regression models to estimate the associations between several life style related risk factors as well as mild analgesic use with cryptorchidism and hypospadia as dependent variables. Maternal and paternal age and BMI were investigated both as categorical and as continuous variable. Medication use was stratified per pregnancy period and evaluated for overall use of analgesics, use of paracetamol, and use of other painkillers. The reference group only contained women who did not use any medication during pregnancy.

Potential confounders were selected based on evidence on relevant determinants of cryptorchidism and hypospadias in an earlier case-referent study conducted in the same area prior to the current birth cohort study.[20] Both maternal and paternal factors were considered. First, we selected all reported potential confounders from both mother and father, including age, educational level, ethnicity, parity, BMI, smoking during pregnancy, alcohol use during pregnancy, underlying diseases, self perceived general health, gestational age at birth, birth weight, occupational exposure to endocrine disruptors, occupational exposure to pesticides, medication use before and during pregnancy, and for women only folic acid supplement use, infectious disease during pregnancy, and fever during pregnancy. Second, maternal age, maternal educational level, maternal BMI, maternal general health, maternal use of co-medication, maternal underlying diseases and maternal fever during pregnancy were included by default,

while the other variables were retained in the multivariable model as confounders when they changed the OR of mild analgesic use by more than 10%.[21] We tested the confounders during the period with the largest OR for mild analgesic use and applied the final set of confounders to the other periods. The final model for both cryptorchidism and hypospadia consisted only of the following confounders which were included by default: maternal age, maternal educational level, maternal BMI, maternal general health, maternal use of co-medication, maternal underlying diseases and maternal fever during pregnancy. Missing values in covariates were handled by multiple imputations (MCMC method) by generating five independent datasets for all analyses. Imputations were based on the relations between all covariates included in this study. The threshold for imputation was 30% of missing values, all covariates were imputed.[22] The pooled estimate from the multivariable models were used to construct the Tables. We carried out a sensitivity analyses to assess the influence of the time enrolment on the effect estimates, comparing women included after 20 weeks of gestation with women enrolled before 20 weeks of gestation. The results from the multivariable analyses were used to estimate the population attributable fraction (PAF), expressing the proportion of the adverse health outcome in the general population that is attributed to exposure to the risk factor of interest. The PAF is a function of both the relative risk and the proportion of exposed persons in the population.[23] In this study ORs were used for the calculations of the PAF.

RESULTS

The cumulative period prevalence of cryptorchidism and hypospadia in our study population were 2.1% and 0.7% respectively. The baseline characteristics of the mothers are shown in Table 1. The univariable and multivariable analyses of the lifestyle related and environmental risk factors are shown in Table 2. We found that being overweight (BMI category 25-30 kg/m^2) was associated with an increased risk of having a child with cryptorchidism. The effects of maternal age (OR per year increase 1.01; 95%CI 0.96-1.06) and BMI (OR per unit kg/m^2 increase 1.04; 95%CI 0.99-1.09) as continuous variables on the occurrence of cryptorchidism were not statistically significant. For hypospadia the effects were close to unity (OR 0.96; 95%CI 0.88-1.04 and OR 1.04; 95%CI 0.95-1.14, respectively). The multivariable analysis showed that after adjusting for use of mild analgesics during pregnancy, the effect estimates of life style and environmental factors were comparable. No other statistically significant differences were present among the groups.

Tables 3 and 4 present the results of the univariable and multivariable analyses on maternal use of mild analgesics during pregnancy and the occurrence of cryptorchidism and hypospadia. In total, 29.9% of the mothers in our study population used mild analgesics during pregnancy (956/3184). There was moderate agreement between mild analgesics users during the distinguished periods with kappa values varying between 0.23 and 0.40.

TABLE 1. Baseline characteristics of the pregnant women (n=3184) included in the analyses

Variables	Cryptorchidism (n=68)	Hypospadia (n=22)	Normal (n=3094)
Maternal characteristics			
Maternal age at intake (years)	30.53 (5.00)	29.07 (5.61)	30.29 (5.12)
Educational level			
Low	18 (26.5%)	6 (27.3%)	669 (21.6%)
Mid	35 (51.5%)	13 (59.1%)	1482 (47.9%)
High	11 (16.2%)	3 (13.6%)	802 (25.9%)
Missing	4 (5.9%)	0	141 (4.6%)
Ethnicity			
Netherlands	32 (47.1%)	12 (54.5%)	1623 (52.5%)
Surinam and Dutch Antilles	7 (10.3%)	0	321 (10.4%)
Morocco and Turkey	10 (14.7%)	6 (27.3%)	437 (14.1%)
Other	15 (22.1%)	4 (18.2%)	615 (10.1%)
Missing	4 (5.9%)	0	98 (3.2%)
Parity			
First child	34 (50.0%)	15 (68.2%)	1710 (55.3%)
Second child or higher	33 (48.5%)	7 (31.8%)	1367 (44.2%)
Missing	1 (1.5%)	0	17 (5.5%)
Conception			
Spontaneous	63 (92.6%)	18 (81.8%)	2892 (93.5%)
Infertility treatment	1 (1.5%)	0	53 (1.7%)
Missing	4 (5.9%)	4 (18.2%)	149 (4.8%)
Health in general			
Good	54 (79.4%)	19 (86.4%)	2566 (82.9%)
Moderate/poor	2 (2.9%)	1 (4.5%)	172 (5.6%)
Missing	12 (17.6%)	2 (9.1%)	356 (11.5%)
Underlying diseases			
No	12 (17.6%)	5 (22.7%)	644 (20.8%)
Yes	41 (60.3%)	14 (63.6%)	1855 (60.0%)
Do not know	4 (5.9%)	2 (9.1%)	309 (10.0%)
Missing	11 (16.2%)	1 (4.5%)	286 (9.2%)
Fever during pregnancy			
No	57 (83.8%)	19 (86.4%)	2571 (83.1%)
Yes	10 (14.7%)	3 (13.6%)	508 (16.4%)
Missing	1 (1.5%)	0	15 (0.5%)
Infection or inflammation during pregnancy			
No	52 (76.55)	15 (68.2%)	2342 (75.7%)
Yes	5 (7.4%)	5 (22.7%)	399 (12.9%)
Missing	11 (16.2%)	2 (9.1%)	353 (11.4%)

TABLE 1. Baseline characteristics of the pregnant women (n=3184) included in the analyses **(continued)**

Variables	Cryptorchidism (n=68)	Hypospadia (n=22)	Normal (n=3094)
Use of co-medication			
No	43 (63.2%)	9 (40.9%)	1734 (56.0%)
Yes	25 (36.8%)	13 (59.1%)	1356 (43.8%)
Missing	0	0	4 (0.1%)
Body Mass Index (kg/m^2)	25.28 (4.75)	25.37 (4.16)	24.54 (4.29)
Missing	0	0	16 (0.5%)
Smoking during pregnancy			
No	46 (67.6%)	17 (77.3%)	2086 (67.4%)
Yes, until pregnancy was known	3 (4.4%)	1 (4.5%)	234 (7.6%)
Yes, after pregnancy was known	7 (10.3%)	2 (9.1%)	449 (14.5%)
Missing	12 (17.6%)	2 (9.1%)	325 (10.5%)
Alcohol use during pregnancy			
No	22 (32.4%)	10 (45.5%)	1271 (41.1%)
Yes, until pregnancy was known	6 (8.8%)	1 (4.5%)	384 (12.4%)
Yes, after pregnancy was known	29 (42.6%)	9 (40.9%)	1132 (36.6%)
Missing	11 (16.2%)	2 (9.1%)	307 (9.9%)
Folic acid use			
No	8 (11.8%)	5 (22.7%)	639 (20.7%)
Yes, post conception start	16 (23.5%)	6 (27.3%)	758 (24.5%)
Yes, preconception start	25 (36.8%)	7 (31.8%)	1015 (32.8%)
Missing	19 (27.9%)	4 (18.2%)	682 (22.0%)
Birth outcomes			
Gestational age at birth	40.4 (33.4-42.9)	39.7 (35.4-42.7)	40.1 (25.3-43.4)
Birth weight	3436.0 (615.6)	3339.3 (739.1)	3491.9 (556.9)

Values are numbers (percentages) for categorical variables, and means (standard deviation) for continuous variables.

Among mothers with cryptorchid sons 33.8% (23 of 68) reported the use of mild analgesics during pregnancy, compared with 31.8% (7 of 22) in mothers with a boy with hypospadia, and 29.9% (926 of 3094) in mothers with healthy boys (adjusted OR 1.25; 95%CI 0.73-2.13 and OR 0.98; 95%CI 0.38-2.52, respectively). Mild analgesics primarily consisted of paracetamol (75%), followed by NSAIDS (13%), and other painkillers such as aspirin (12%).

A total of 484 (20.8%) and 252 (10.9%) mothers reported the use of mild analgesics during the periconception period and the first period, respectively. Use of mild analgesics in the periconception and first period was not associated with cryptorchidism or hypospadia (adjusted OR 0.89; 95%CI 0.42-1.88, OR 0.94; 95%CI 0.36-2.46 for cryptorchidism, adjusted OR 1.36; 95%CI 0.47-3.94, OR 2.05; 95%CI 0.64-6.58 for hypospadia).

TABLE 2. Univariable and multivariable logistic regression of maternal characteristics and life style related factors and the association with cryptorchidism and hypospadia

Variables	Cryptorchidism	Hypospadia	Cryptorchidism	Hypospadia
	OR (95%CI)	OR (95%CI)	aOR (95%CI) [a]	aOR (95%CI) [a]
Maternal age at intake				
<25 years	1.00	1.00	1.00	1.00
25-29 years	1.28 (0.57-2.88)	0.43 (0.12-1.52)	1.28 (0.57-2.86)	0.43 (0.12-1.52)
30-35 years	1.37 (0.64-2.90)	0.64 (0.23-1.79)	1.35 (0.63-2.87)	0.63 (0.22-1.78)
>35	1.34 (0.56-3.20)	0.50 (0.13-2.02)	1.32 (0.55-3.16)	0.50 (0.12-2.00)
Educational level				
Low	1.96 (0.92-4.18)	2.40 (0.60-9.62)	1.91 (0.89-4.10)	2.32 (0.58-9.34)
Mid	1.72 (0.87-3.41)	2.35 (0.67-8.25)	1.78 (0.91-3.49)	2.31 (0.66-8.12)
High	1.00	1.00	1.00	1.00
Ethnicity				
Netherlands	1.00	1.00	1.00	1.00
Surinam and Dutch Antilles	1.11 (0.48-2.53)	-	1.09 (0.48-2.48)	-
Morocco and Turkey	1.16 (0.57-2.38)	1.86 (0.69-4.98)	1.12 (0.55-2.31)	1.84 (0.68-4.96)
Other	1.24 (0.67-2.30)	0.88 (0.28-2.74)	1.33 (0.70-2.51)	0.88 (0.28-2.75)
Parity				
First child	1.00	1.00	1.00	1.00
Second child or higher	1.21 (0.75-1.97)	0.58 (0.24-1.44)	1.18 (0.73-1.91)	0.58 (0.24-1.43)
Health in general				
Good	1.00	1.00	1.00	1.00
Moderate/poor	0.55 (0.13-2.29)	0.79 (0.10-5.90)	0.60 (0.12-2.96)	0.72 (0.10-5.35)
Underlying disease				
No	1.00	1.00	1.00	1.00
Yes	1.19 (0.62-2.27)	0.97 (0.35-2.71)	1.07 (0.57-2.00)	1.03 (0.37-2.88)
Fever during pregnancy				
No	1.00	1.00	1.00	1.00
Yes	0.89 (0.45-1.75)	0.80 (0.24-2.71)	0.86 (0.44-1.72)	0.78 (0.23-2.68)
Inflammation or infection during pregnancy				
No	1.00	1.00	1.00	1.00
Yes	0.56 (0.22-1.42)	1.96 (0.71-5.41)	0.63 (0.24-1.66)	1.84 (0.67-5.08)
Use of co-medication				
No	1.00	1.00	1.00	1.00
Yes	0.74 (0.45-1.22)	1.85 (0.79-4.33)	0.70 (0.42-1.17)	1.87 (0.78-4.48)
Body Mass Index				
<25 kg/m^2	1.00	1.00	1.00	1.00
25-30 kg/m^2	1.83 (1.08-3.10)*	2.18 (0.90-5.29)	1.81 (1.07-3.07)*	2.17 (0.90-5.27)
>30 kg/m^2	1.44 (0.69-3.03)	1.02 (0.23-2.63)	1.45 (0.69-3.03)	1.02 (0.23-4.63)

TABLE 2. Univariable and multivariable logistic regression of maternal characteristics and life style related factors and the association with cryptorchidism and hypospadia **(continued)**

Variables	Cryptorchidism	Hypospadia	Cryptorchidism	Hypospadia
	OR (95%CI)	OR (95%CI)	aOR (95%CI) [a]	aOR (95%CI) [a]
Smoking during pregnancy				
No	1.00	1.00	1.00	1.00
Yes, until pregnancy was known	0.58 (0.18-1.88)	0.52 (0.07-3.96)	0.57 (0.19-1.76)	0.48 (0.06-3.60)
Yes, after pregnancy was known	0.71 (0.32-1.58)	0.55 (0.13-2.37)	0.74 (0.34-1.62)	0.58 (0.13-2.53)
Alcohol use during pregnancy				
No	1.00	1.00	1.00	1.00
Yes, until pregnancy was known	0.90 (0.36-2.24)	0.33 (0.04-2.59)	0.69 (0.28-1.65)	0.27 (0.04-2.09)
Yes, after pregnancy was known	1.48 (0.85-2.59)	1.01 (0.41-2.50)	1.15 (0.68-1.95)	0.84 (0.35-2.00)
Folic acid use				
No	0.51 (0.23-1.13)	1.14 (0.36-3.59)	0.75 (0.37-1.51)	1.17 (0.38-3.58)
Yes, post conception start	0.86 (0.45-1.62)	1.15 (0.38-3.43)	0.82 (0.40-1.65)	1.29 (0.39-4.32)
Yes, preconception start	1.00	1.00	1.00	1.00

[a] Adjusted for maternal use of mild analgesics during pregnancy.

In the second period (14-22 weeks of gestation), containing 2864 women, 480 (16.8%) women reported the use of mild analgesics. Use during the second period in particular increased the risk of congenital cryptorchidism (adjusted OR 2.12; 95%CI 1.17-3.83). The risk remained statistically significant for the individual compound paracetamol (adjusted OR 1.89; 95%CI 1.01-3.51). Associations with hypospadia were not observed.

In the third period (20-32 weeks of gestation), 363 out of 2709 (13.4%) women reported the use of mild analgesics. Maternal use of mild analgesics during the third period was not associated with congenital cryptorchidism or hypospadia (adjusted OR 1.56; 95%CI 0.78-3.11, and OR 0.32; 95%CI 0.04-2.44, respectively).

The results of the sensitivity analyses indicated that there was no change in the effect estimates when we restricted our analysis to women included before 20 weeks of gestation, however, due to the smaller sample size, the confidence intervals widened.

The PAFs for the use of mild analgesics in the second and third trimester varied between 0.09 and 0.24 (95%CI 0.00-0.29, and 0.04-0.43, respectively).

TABLE 3. Univariable logistic regression of maternal use of mild analgesics during pregnancy and the association with cryptorchidism and hypospadia

Use of painkillers	Cryptorchidism		Hypospadia		Normal
	n (%)	OR crude (95%CI)	n (%)	OR crude (95%CI)	n (%)
Periconception period (n=2322 boys)					
Use of mild analgesics during the periconception period	9 (19.1%)	0.90 (0.43-1.88)	5 (27.8%)	1.46 (0.52-4.12)	470 (20.8%)
Specific substances					
Paracetamol	7 (14.9%)	0.99 (0.44-2.23)	3 (16.7%)	1.24 (0.35-4.37)	333 (14.8%)
Other painkillers	2 (4.3%)	0.69 (0.16-2.88)	2 (11.1%)	2.01 (0.45-8.98)	137 (6.1%)
First period (n=2090 boys)					
Use of mild analgesics during the first period	5 (10.6%)	0.97 (0.38-2.48)	4 (22.2%)	2.26 (0.73-7.00)	243 (10.8%)
Specific substances					
Paracetamol	5 (10.6%)	1.34 (0.52-3.44)	3 (16.7%)	2.34 (0.66-8.30)	176 (7.8%)
Other painkillers	0	-	1 (5.6%)	2.05 (0.27-15.91)	67 (3.0%)
Second period (n=2864 boys)					
Use of mild analgesics during second period	17 (28.8%)	2.04 (1.15-3.62)*	2 (10.0%)	0.56 (0.13-2.42)	461 (16.6%)
Specific substances					
Paracetamol	15 (25.4%)	1.85 (1.02-3.37)*	2 (10.0%)	0.58 (0.13-2.49)	448 (16.1%)
Other painkillers	2 (3.4%)	8.51 (1.86-38.91)*	0	-	13 (0.5%)
Third period (n=2709 boys)					
Use of mild analgesics during third period	11 (19.3%)	1.56 (0.80-3.03)	1 (5.3%)	0.36 (0.05-2.71)	351 (13.3%)
Specific substances					
Paracetamol	11 (19.3%)	1.62 (0.83-3.16)	1 (5.3%)	0.38 (0.05-2.83)	337 (12.8%)
Other painkillers	0	-	0	-	14 (0.5%)

TABLE 4. Multivariable analysis of maternal use of mild analgesics during pregnancy and the association with cryptorchidism and hypospadia

Use of painkillers	Cryptorchidism	Hypospadia
	OR adjusted[1] (95%CI)	OR adjusted[2] (95%CI)
Use of mild analgesics during the periconception period	0.89 (0.42-1.88)	1.36 (0.47-3.94)
Specific substances		
Paracetamol	1.02 (0.44-2.36)	1.19 (0.33-4.32)
Other painkillers	0.61 (0.14-2.58)	1.71 (0.37-7.81)
Use of mild analgesics during the first period	0.94 (0.36-2.46)	2.05 (0.64-6.58)
Specific substances		
Paracetamol	1.38 (0.52-3.64)	2.24 (0.60-8.32)
Other painkillers	-	1.65 (0.21-13.08)
Use of mild analgesics during second period	2.12 (1.17-3.83)*	0.53 (0.12-2.34)
Specific substances		
Paracetamol	1.89 (1.01-3.51)*	0.54 (0.12-2.41)
Other painkillers	8.93 (1.84-43.24)*	-
Use of mild analgesics during third period	1.50 (0.75-3.00)	0.31 (0.04-2.35)
Specific substances		
Paracetamol	1.56 (0.78-3.11)	0.32 (0.04-2.44)
Other painkillers	-	-

[1] Adjusted for maternal age, educational level, BMI at intake, general health, use of co-medication, underlying diseases, and fever during pregnancy.
[2] Adjusted for maternal age, educational level, BMI at intake, general health, use of co-medication, underlying diseases, and fever during pregnancy.
* p value < 0.05.

DISCUSSION

This large population-based prospective cohort study suggests that maternal exposure to mild analgesics during the second period (reflecting gestational weeks 14-22) in pregnancy is associated with an increased prevalence of cryptorchidism in the offspring, whereas no associations were observed during the first and third period of pregnancy. However, we must note that the current study has several limitations, most important the limited number of cases of cryptorchidism and hypospadia. We must also note that the definition of the different pregnancy periods was based on the average response time to the questionnaires, which will result in some lack of precision of the exact duration of the period. The distinguished pregnancy periods necessarily overlap, since standardised questions with fixed recall periods were used. Also, the users of analgesics in the different trimesters partly overlapped (kappa values 0.23 to 0.40), which cannot exclude the possibility of a contribution of the previous trimester to the internal dose in the subsequent trimester. Despite these limitations, our findings suggest that intrauterine exposure to mild analgesics during pregnancy might increase the risk of cryptorchidism. Since the

proportion of women using mild analgesics during pregnancy is high, the population impact may be substantially. This observation corroborates findings from experimental rat studies and two recent observational studies.

The strength of this study is the population-based approach with recruitment during the prenatal period which enabled us to assess, and adjust for, a large number of potential confounders. Since smoking, alcohol use, and folic acid supplement use had many missing values, we used multiple imputation to handle missing values in our covariates. This reduces selection bias due to non-random missing in the covariates. Another strength of the study lies in the frequent assessments that took place in the child health care centres, with assessments for cryptorchidism during five screening visits in the first six months. Spontaneous testicular descent occurs in up to 75% of cryptorchid testis during the first three months of life when reproductive hormone activity is high.[24] The majority of children in our study visited the child health care centres in the first six months (89,4%), and subsequent visits made it possible to examine children who did not visit the child health care centre between 0-6 months, resulting in a cumulative period prevalence. We used questionnaires to assess the exposure to paracetamol and other mild analgesics. These analgesics are bought over-the-counter and therefore it was not possible to check information from pharmacies or general practitioners. We explicitly asked for use of painkillers which can be bought over-the-counter, and we observed that the number of women who reported use of mild analgesics in our study (29.9%) was comparable to Danish women reporting mild analgesic use in self-administered questionnaires in the study of Kristensen et al. (26.1% and 30.9%). In our cohort information on medication use was collected prior to the information on congenital malformations, which were diagnosed after birth and, thus, recall bias is unlikely. Although misclassification may have occurred, we believe that this is most likely non-differential misclassification, leading to an underestimation of the observed effect estimates. An analysis restricted to women included before 20 weeks of gestation showed very similar results.

Some other limitations need to be addressed. Selection bias due to non-response would be present if the association of medication use with cryptorchidism or hypospadia differed between those with (n = 3184) and those without (n = 524) information on medication use. Although the general characteristics of women with data on medication use were slightly different from those without data on medication use, no difference in the occurrence of cryptorchidism and hypospadia was observed. Thus, selection bias due to non-response on medication use seems unlikely but cannot be excluded.

Another shortcoming of our design is the lack of information on the frequency and dose of painkiller use. Also, given the overlap between analgesics users in different periods during pregnancy, it is difficult to ascertain with certainty that mild analgesics use during one specific period is associated with an increased risk of cryptorchidism. However, agreement between the different periods during pregnancy was calculated by means of Kappa values, which ranged between 0.23 to 0.40, indicating low agreement between these different pregnancy periods.

Despite this suboptimal design, we showed an association between use of mild analgesics during the second period in pregnancy and cryptorchidism. This study could increase the awareness for this important topic and stimulate researchers to set up larger studies.

We calculated population attributable fractions, ranging from 9%-24%, which suggest, if causality could be established, that at best about 24% of all cases of cryptorchidisms in our study population could be attributed to use of mild analgesics during pregnancy. This illustrates that for the majority of cryptorchidism cases other causes must be responsible, such as suboptimal maternal health, low birth weight, and small-for-gestational-age.[20,25]

Women who use painkillers during pregnancy may suffer from an underlying disorder which prompts medication use. The majority of women in our study used the analgesics for common pain such as headache and muscle ache. Confounding by indication may be present in mothers who regularly use painkillers, but adjustment for underlying diseases did not change the effect estimates by more than 10%. The analyses were adjusted for general health, use of co-medication, underlying diseases and fever during pregnancy as important proxy variables for indication to treat. None of the paternal risk factors in this study changed the association between maternal use of mild analgesics and cryptorchidism or hypospadia with more than 10%, thus paternal risk factors were not considered to be potential confounders. The study by Pierik et al. showed various paternal risk factors associated with the occurrence of cryptorchidism,[20] however, in the current study these paternal risk factors did not confound the association between mild analgesic use of the mother during pregnancy and the occurrence of cryptorchidism. These paternal risk factors seem independent risk factors for cryptorchidism, but were not associated with the use of mild analgesics of the mother during pregnancy. However, residual confounding cannot be completely ruled out.

Cryptorchidism and hypospadia are among the most frequent congenital abnormalities in male births.[2] Maternal life style factors and environmental exposures during pregnancy are suspected to interfere with the normal testicular decent and possibly increase the risk of cryptorchidism.[3,26] Experimental rat models have indicated that the testis decent from the intra-abdominal position into the scrotum takes place in two phases, a transdabdominal phase and an inguinoscrotal phase.[12,27] However, in humans, discussion exists over the exact time window in which the testis descent takes place. Hutson et al. described that the transdabdominal phase occurs between 8-14 weeks of gestation,[27] whereas Foresta et al. defines this phase between 10-23 weeks of gestation.[25] Further research is needed to identify the exact critical time window in which testicular descent takes place.

Impairment of normal androgen action, through exposure to endocrine disrupting chemicals or medication in these crucial time windows, is associated with adverse reproductive developmental endpoints in experimental animals.[28] Recently, Kristensen et al. showed that paracetamol, even at low plasma concentrations of 1 μM, is a potent inhibitor of testosterone production, resulting in impaired masculinisation in rat models.[9] These findings are also confirmed by two observational studies that showed similar associations between use of mild

analgesics and cryptorchidism, in particular for the second trimester.[9,16] We did not find an association between use of mild analgesics during pregnancy and hypospadia in the offspring, however we had very few cases (n = 22) of hypospadia in our study. Therefore, we were unable to study with sufficient discriminatory power the hypothesised underlying mechanism of impairment of normal androgen action, resulting in abnormal sexual differentiation, for hypospadias, and the origin of hypospadia, genetic, endocrine or environmental remains unclear.[29]

Mild analgesics such as NSAIDs act by inhibiting cyclooxygenases COX 1 and 2. Acetaminophen (paracetamol) is thought to block the peroxidase function of COX enzymes,[30] although the precise mechanism by which the drug exerts its action is still uncertain.[31] COX enzymes catalyse a key step of the synthesis of prostaglandins from arachidonic acid. It has been shown that the testosterone-dependent differentiation of the male reproductive tract requires the continuing synthesis of prostaglandins.[32] Suppression of prostaglandin production by interfering with the arachidonic acid cascade at the level of release of arachidonic acid from cell membrane lipids or by inhibiting COX enzymes diminishes foetal androgen action and compromises male sexual differentiation.[33] Accordingly, Kristensen et al. demonstrated that acetaminophen is capable of suppressing foetal androgen synthesis in male rats exposed ex vivo to the drug during late foetal life. The day before birth the male foetuses showed reduced anogenital distances, another sign of diminished androgen action.[9] In a subsequent paper, Kristensen et al. were able to pinpoint the suppression of prostaglandin synthesis by acetaminophen and other NSAIDs to the inhibition of COX enzymes.[34] Importantly, the same authors revealed many other putative endocrine disrupting chemicals, including phthalates and other phenolic agents as possessing prostaglandin-inhibitory potential. In the light of these observations, it appears biologically plausible that not only NSAIDs, but also other endocrine disrupting chemicals may contribute to increasing the risks of developing cryptorchidism by interfering with prostaglandin synthesis. It becomes necessary to address this possibility in further epidemiological studies.

In conclusion, we found an association between maternal use of mild analgesics, in particular paracetamol, during the second period in pregnancy (14-22 weeks of gestation) and congenital cryptorchidism. Since the number of women using mild analgesics during pregnancy is high (approximately 30-40%) further research is urgently needed to corroborate these findings, so that preventive measures, if needed, can be taken.

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PART 5

GENERAL DISCUSSION

1. INTRODUCTION

Workplace health is an important issue, since women who intend to become pregnant and pregnant women are at risk for several reproductive outcomes, thus, it is important to identify occupational risk factors for prevention. Although women in paid employment generally have a better reproductive health than those without paid employment, certain work-related risk factors, such as exposure to chemicals, physically demanding work, and psychological job strain, may influence women's reproductive abilities. In this thesis, several studies are presented that focus on occupational risk factors, in particular exposure to chemicals and physically demanding work, and its association with several aspects of reproductive health, such as fecundity, intrauterine growth, pregnancy complications and congenital malformations. Insight in these issues is important in order to improve the clinician's ability to counsel couples who are trying to conceive or women who have concerns about their pregnancy.

As described in the introduction, the primary objectives of this thesis are:

1. To study the influence of occupational exposure to chemicals on reproduction, specifically fecundity, intrauterine growth, hypertensive disorders during pregnancy, and birth outcomes.
2. To study the influence of physically demanding work on intrauterine growth, hypertensive disorders during pregnancy, and birth outcomes.
3. To study the relation between exposure to endocrine disruptors (EDs) and the occurrence of congenital malformations, including congenital heart defects (CHDs) and male reproductive tract abnormalities, such as cryptorchidism and hypospadia.

This chapter will present the main findings from this thesis. Furthermore, methodological issues will be discussed and new insights and directions for future research will be presented.

2. SUMMARY OF THE MAIN FINDINGS

Study aim 1; chemicals and reproduction

Our first study aim addresses occupational exposure to chemicals in relation to various aspects of human reproduction, including fecundity, intrauterine growth, hypertensive disorders, and birth outcomes. Since 1970, epidemiologic research has demonstrated several causal relationships and many possible associations between environmental exposures and adverse pregnancy and adult health concerns.[1] Disorders of reproduction and hazards to reproductive health and associated functions have become prominent issues in recent decades after reports of adverse effects of several chemicals on reproductive function.[2]

Chemicals, endocrine disruptors and fecundity

Factors related to postponement of motherhood,[3,4] smoking,[5,6] and alcohol or caffeine intake[7] may interfere with the reproductive system.[1] However, the attention has grown for work-related and environmental factors which may also reduce fertility.[8] In the early 1990s, studies began to associate environmental contaminants with altered reproductive performance in wild populations of fish, amphibians, reptiles and birds.[9] Together with concerning trends in human reproductive health, such as the rising incidence in testicular cancer,[10] and low average sperm counts,[11] this led to the hypothesis that chemical contaminants may negatively affect the reproductive process causing reduced fertility and adverse pregnancy outcomes in the general population. With a systematic review we aimed to summarise the existing literature on exposure to chemicals and time to pregnancy (TTP), as a measure of couple's fecundity (**Chapter 2.1**). From this review we can conclude that there are strong indications that certain occupational exposures, such as pesticides and lead, adversely influence male and female fertility. These conclusions are in line with earlier reviews on pesticide exposure and fertility.[12-14] Regarding occupational exposure to lead, the evidence is quite consistent, showing that lead exposure reduces fertility and prolongs TTP.[15,16] This was further substantiated by findings within the Generation R cohort (**Chapter 2.2**). We showed that paternal occupational exposure to polycyclic aromatic hydrocarbons (PAHs), heavy metals and overall exposure to chemicals with endocrine disrupting properties was associated with a prolonged TTP. For pesticides, we found for both maternal and paternal occupational exposure decreased hazard ratios, however, these were not statistically significant, probably due to the low prevalence of exposure to pesticides.

Chemicals and foetal growth

Several studies from the 1990s onwards found that risk factors for foetal development, such as poor maternal nutrition, can results in an increased risk of adult onset of chronic conditions such as coronary heart disease.[17,18] These findings led to the foetal origins of disease hypothesis (commonly known as the 'Barker Hypothesis'), which proposes that exposures to adverse

insults during critical or sensitive windows of development can permanently reprogram normal physiologic responses, and thus give rise to illnesses and metabolic and hormonal disorders later in life.[19,20] Two large reviews have summarised the epidemiologic literature on exposure to environmental contaminants during pregnancy and adverse birth outcomes, such as low birth weight, intrauterine growth retardation (IUGR), and preterm delivery,[21,22] suggesting a variety of links. Suggestive evidence associates pesticides and polychlorinated biphenyls with decreased foetal growth and pregnancy length. Further studies in a large birth cohort in Sweden on parental occupation in relation to foetal growth and pregnancy length gave further rise to the hypothesis that occupational exposures may influence foetal growth.[23,24] The articles described in this thesis focus on the impact of maternal exposure to chemicals during pregnancy on pregnancy outcome. Since various birth outcomes have been studied extensively in relation to chemical exposure, and birth outcomes are a rather crude marker of intrauterine circumstances, we focussed on the effects of chemicals on intrauterine growth.[25-30] The study on occupational exposure to chemicals, suggests that maternal occupational exposure to PAHs, phthalates, alkylphenolic compounds, and pesticides adversely influenced several domains of foetal growth during pregnancy and also adversely influenced placental weight (**Chapter 2.3**). This study supported existing evidence from human studies regarding occupational exposure and adverse pregnancy outcomes.[1]

Measurement strategy is becoming more important the last decades, and the characterisation of exposure with the Job-Exposure-Matrix (JEM) must be interpreted with caution, since this is a rather crude measure of exposure. Biomonitoring data on occupational exposures is rare, but would be a step forward for a better exposure assessment strategy. With the study on prenatal exposure to bisphenol A (BPA), we examined the effects on urinary concentrations of BPA on intrauterine growth, and we were able to study whether a single urinary measurement is a good proxy for exposure to BPA, or whether we would prefer to measure two or even three samples during pregnancy. In this study, we showed that higher concentrations of creatinine-based BPA in prenatal urine were associated with a slower foetal growth rates for both foetal weight and head circumference (**Chapter 2.4**). Most importantly, this study clearly showed, that the number of measurements per individual strongly influenced the effect estimates for foetal head circumference and foetal weight. When fewer measurements were used these estimates were close to unity, and when three available measurements were used, the estimates were highly statistically significant. The BPA-foetal growth relation may fit the profile of a setting where, for a fixed total number of measurements, more replicates and fewer subjects maximises power.[31]

Study aim 2; physically demanding work and reproduction

The risks of physically demanding work during pregnancy on foetal growth, adverse birth outcomes and hypertensive pregnancy complications are addressed in study aim 2. Occupational risk factors, such as working in a specific occupation,[32,33] shift work,[34,35] job stress,[36-38] standing,

lifting,[39] and work hours[40-42] have been related to adverse birth outcomes. Two reviews have suggested an influence of physically demanding work on pregnancy outcomes, albeit of modest magnitude.[43,44] In the study on physically demanding work and foetal growth, we were unable to demonstrate clear adverse effects of physically demanding work and working hours on adverse birth outcomes. However, effects of prolonged standing on foetal head circumference and long working hours on both foetal head circumference and foetal weight could be demonstrated in the longitudinal analyses (**Chapter 3.2**). Furthermore, we studied the effects of physically demanding work, working hours, and exposure to chemicals on hypertensive pregnancy complications (**Chapter 3.1**). This study suggests that there was no influence of physically demanding work and exposure to chemicals on hypertensive disorders during pregnancy. However, the low prevalence of hypertensive disorders during pregnancy combined with the low prevalence of occupational risk factors limit the conclusions and larger studies are needed to corroborate these findings.

Study aim 3; endocrine disruptors and congenital anomalies

Chemicals and congenital malformations

Congenital malformations are the leading cause of infant morbidity, accounting for more than 20% of all infant deaths, and congenital heart defects (CHDs) constitute the largest group of congenital anomalies, accounting for nearly 30% of children with major congenital anomalies diagnosed prenatally or in infancy in Europe.[45] During the past 20 years, environmental risk factors for human birth defects have drawn attention from the public and scientific communities.[46] Epidemiological evidence of associations between occupational exposure to chemicals and CHDs is scarce and contradictory.[46-49] Since prospective cohort studies are difficult because of the low prevalence of CHDs, case-control studies with a standardised postnatal data collection are the best alternative. In this study we found an association between occupational exposure of the father to specific chemicals and an increased risk of CHDs (**Chapter 2.5**).

Mild analgesics and the occurrence of cryptorchidism and hypospadia

Two large cohort studies in Denmark found an association between the use of mild analgesics during pregnancy with the occurrence of cryptorchidism in the offspring,[50,51] and gave rise to the hypothesis that mild analgesics could influence the androgen dependent descent of the testis.[52] Normal androgen action during the critical programming window of testis descent is crucial for the descent of the testis, and factors that diminish androgen action during that time,[53] such as paracetamol, have detrimental consequences for male sexual differentiation. We were able to substantiate these findings and found an association between maternal exposure to mild analgesics during the second trimester in pregnancy with an increased prevalence of cryptorchidism (**Chapter 4.1**).

Summary

The studies described in **Part 2** show that occupational exposure to chemicals adversely influenced the reproductive abilities of women. Furthermore, some indications for effects of physically demanding work on intrauterine growth were found in **Part 3**, however, no effects of physically demanding work and exposure to chemicals were found on hypertensive pregnancy complications. In **Part 4** we demonstrated that use of mild analgesics during pregnancy may influence the occurrence of cryptorchidism in the offspring. We schematically summarised our main findings in Table 1.

TABLE 1. Schematic overview of the described findings in the studies on occupational risk factors in relation to reproductive health

Exposure	Fertility	Hypertensive complications	Foetal growth	Adverse birth outcomes	Congenital Malformations
Maternal chemical exposure	No effect	No effect	↓ FW/HC/FL	n.a.	No effect
Paternal chemical exposure	↑ TTP				↓ CHDs
Physically demanding work		No effect	↓ HC	No effect	
Long working hours		No effect	↓ FW/HC	No effect	
Mild analgesics					↑ CRYPT

TTP, time to pregnancy; FW, foetal weight; HC, head circumference; FL, foetal length; CHD, congenital heart defect; CRYPT, cryptorchidism; n.a., not in this thesis.

3. METHODOLOGICAL CONSIDERATIONS

The studies described in this thesis have mainly been conducted within the Generation R Study, a population-based prospective cohort study. In such a study, groups of individuals who are alike in many ways but differ by a certain characteristic, are classified according to an exposure, followed over time, and compared for a particular outcome.[54]

Observational prospective studies have specific strengths and limitations. Among the strengths of cohort studies are that they provide incidence data, they assess temporal relationships between exposure and effect, they can measure and subsequently adjust for a broad set of confounding variables, and they can measure multiple outcomes. There are also some limitations to cohort studies, including that they are time-consuming and expensive, they cannot study rare outcomes, and they need a lot of manpower. Furthermore, they are sensitive to bias that may threaten the internal validity; these include selection bias, information bias and confounding. However, experimental studies are unfeasible and unethical for many of the research topics described in this thesis. The extent to which the results presented in this thesis are influenced by these types of bias will be discussed in the next paragraphs.

3.1 Assessment of exposure and outcome

Occupational exposure to chemicals

The ideal method for assessing occupational or environmental exposures of subjects in epidemiological studies is quantitative measurement of external concentrations in the air or on the skin, or measurement of internal dose in body tissues or other human material. Unfortunately, in many study designs, this ideal is difficult or impossible to achieve. JEMs are used as surrogate exposure measures next to the occupation or industry as proxy for exposure. JEMs list occupation and/or industries on one axis, and exposure agents on the other, and the cells of the matrix indicate the presence, intensity, frequency, and/or probability of exposure to a specific agent in a specific job.

The JEM for EDs used in this thesis has several limitations. The characterisation of exposure in the JEM must be interpreted as an exposure probability, which is only a crude measure of exposure. Furthermore, the JEM does not contain specific chemicals, but only broad groups of chemicals, and mechanisms of action may vary between specific chemicals in a group. Another major drawback is that JEMs do not account for variability in tasks and working environments within job titles. But, from the task description it may become clear that some subjects within a specific job title are less likely to be exposed to certain chemicals. If misclassification occurs, this will most likely be non-differential misclassification, since exposure status was blinded to participants and researchers.

We observed that the exposure prevalence for occupational exposure to chemicals was very low. In some situations this has resulted in lack of power to detect associations. For example, in the study on occupational exposure to EDs and TTP (**Chapter 2.2**) we found an effect of maternal exposure to pesticides on TTP. However, due to the small number of women exposed (n = 15) the resulting hazard ratio was not significant (HR of 0.62; 95%CI 0.34-1.12).

An alternative for JEMs is biomonitoring of chemicals in body fluids, such as maternal blood or urine. But feasibility issues, costs, and time may restrict biological monitoring in epidemiological studies and it is rare that biological samples can be obtained from the entire study population, particularly in large epidemiological studies. In **Chapter 2.4** we described prenatal exposure to BPA measured in maternal urine in relation to foetal growth. BPA was only measured in 220 women, mainly due to high costs for determination of these chemicals in urine. Within Generation R, we collected urine samples between February 2004 and November 2005 of a part of our study population, response rates varied between 85-97% of eligible women, generally a selection of women mainly from Dutch origin, with higher education and less life-

style related risk factors. Although we randomly selected urine samples from this population, selection effects may have occurred, since the number of women in the analyses is small.

Epidemiological exposure-response analysis of chemicals, such as BPA, is complicated by the fact that exposure to chemicals may occur in mixtures and it may therefore be difficult to single out and attribute specific health effects to a specific agent. Within the JEM we noticed that maternal occupational exposure to phthalates, organic solvents and alkylphenolic compounds were interrelated (Kappa values 0.47-0.77). Thus, it was impossible to disentangle the specific role of certain chemicals. Among fathers there was little overlap in the exposure categories. In **Chapter 2.2** we also investigated the agreement between maternal and paternal occupational exposure to chemicals, and we found that there was little overlap illustrated by Kappa values ranging between 0.03 and 0.13. Mutual adjustments did not change the effect estimates, thus, residual confounding by exposure pattern of the partner could be largely ruled out.

Background exposure to various chemicals through diet and environment may occur. Previous research within the Generation R Study[55] showed that almost all pregnant women are exposed to a variety of chemicals, and that levels are comparable between pregnant and non-pregnant women.[56] However, there is reason to believe that occupational exposure is generally much higher than background exposure through diet and environment.[57] For example, for phthalates, Hines et al. showed that in several occupations the urinary phthalate concentrations exceeded the levels of the general population.[58] However, biomonitoring data comparing occupational exposures with exposure from non-occupational sources are scarce. In this thesis we did not assess background exposure and, thus, it is not possible to distinguish the importance of different routes of exposure. Since it is unlikely that the widespread environmental exposure is associated with occupational exposure in specific jobs, background exposure will most likely not confound the observed relation between occupational chemical exposure and various reproductive outcomes.

Physically demanding work

A large review by Bonzini et al. concludes that a limitation of the available evidence on physically demanding work and adverse pregnancy outcomes is related to the definition and ascertainment of exposure.[44] Many of the occupational activities studied are complex constructs, and cannot be characterised by a simple, undimensional metric. In **Chapter 3.1** and **3.2** we described two studies on physically demanding work and long working hours in relation to pregnancy complications and pregnancy outcome. A limitation of these studies is the semi-quantitative nature of the exposure information in four self-reported categories. This did not allow us to investigate duration of standing and walking per week or frequency of lifting heavy weights. For example, for occupational lifting the following aspects might be important for classification of exposure: frequency of lifting tasks in a working day, the duration of such tasks, the heaviness of the weights lifted, and perhaps also the postures in which lifting is carried out. Exposure was ascertained by a questionnaire during mid-pregnancy. The accuracy of

self-reported data is likely to differ according to their nature. For example, hours of work and night work should be relatively easy to recall, whereas frequency of lifting may be more difficult to remember. Unfortunately, the information collected by questionnaire in Generation R was insufficiently accurate to allow clear counseling of pregnant women working in physically demanding jobs.

Exposure to mild analgesics

The major shortcoming in the design of this study was the lack of information on the frequency, dosage and specific time period of painkiller use. The assessment by questionnaire, where we specifically asked for over-the-counter self medication, showed that 29.9% of the women in our study used mild analgesics during pregnancy, which was comparable to Danish women reporting mild analgesic use by self-administered questionnaire in the study of Kristensen et al.[51]

Assessment of TTP

Assessment of TTP was by self-administered questionnaire during mid-pregnancy, which included a question on the natural origin of the pregnancy (yes/no) and, in case of a positive answer, women with a planned pregnancy were asked about the number of months it took the couple to conceive. Refusal to answer these questions is rare, as this question is readily accepted in a wide range of cultures.[59] Validation studies of TTP have shown that self-reports on TTP give an accurate representation of the true TTP distribution.[60,61] To investigate common biases related to answering the TTP questions, such as wantedness bias and pregnancy planning bias, we carried out several sensitivity analyses, and these analyses indicated little evidence for the presence of these biases.

Assessment of foetal growth and hypertensive pregnancy complications

The ultrasound measures used for the determination of foetal growth were also used for pregnancy dating in the first trimester of pregnancy, since a large proportion of women in the Generation R Study did not have a regular cycle and certain date of the last menstrual period. A disadvantage of pregnancy dating by ultrasound is that growth variation in crown-rump length and biparietal diameter in early pregnancy are assumed to be zero, impairing detailed analyses on foetal growth in the first trimester. Examining foetal growth characteristics instead of birth weight is a more appropriate approach to assess the effects of occupational and environmental risk factors, since it enables identification of specific critical periods during pregnancy for the influence of exposure on patterns of foetal growth and development. However, due to the use of ultrasound measurements for pregnancy dating, we were unable to assess whether these risk factors influenced early growth during the first trimester. In **Chapter 3.1** strict criteria were used to assess hypertensive complications during pregnancy. Medical records were checked and the diagnosis was made by qualified medical doctors. The low prevalence of

these disorders in this study can be explained by the strict criteria for diagnosis. The very low prevalence of pregnancy induced hypertension and preeclampsia in this study, combined with the low prevalence of occupational risk factors, may have resulted in too little discriminatory power to detect associations.

Assessment of congenital heart defects

Children diagnosed with a CHD in the first 15 months after birth by a pediatric cardiologist were identified from the hospital registry and invited to participate. Diagnoses were confirmed by echocardiography and/or catheterisation and/or surgery. The study moment of 15 months after child birth reduces misclassification in the selection of children with and without CHD. Since most congenital malformations are diagnosed in the first year of life, this approach assured including most children with CHD in the study. Children who died of CHD before 15 months are not included in the study population, which may have led to some selection in the severity of the included CHDs.

Assessment of reproductive disorders

The presence of cryptorchidism and hypospadia was assessed during routine screening assessments performed in child health care (CHC) centres, and 93.2% of the children eligible for our current study visited the child health care centres. In an earlier study by Pierik et al. on the prevalence of cryptorchidism and hypospadia in Rotterdam, physicians were trained to perform standardised examinations of the male genitalia.[62,63] During the course of the study, new CHC physicians were also instructed on the standardised examination, and every six months a meeting with the CHC physicians was organised to re-inform the physicians on the study procedures. Due to this extensive training, we believe that the assessment of both disorders was fairly accurate in the current study in Generation R.

3.2 Interpretation in statistical analyses

Missing data frequently occurs in follow up studies. The proper method to handle missing values is dependent on the type of missings. An impropriate method to handle missing values can threat the validity of the study. There are three types of missings: missing completely at random, missing at random, and missing not at random. Missing completely at random means that missing data is completely due to coincidence, for example when due to logistic problems, i.e. questionnaires were not sent in a certain period. Missing at random is present when missings are related to variables in the study, for example a specific group of people has more often missing values, but this is not related to the outcome under study. Missing not at random occurs when missings are associated with both determinant and outcome, for example children from low-income families having more overweight do not show up at the child health centres. There is no statistical analysis that can test what kind of missings one is dealing with, however, it is possible to detect different types of missings when characteristics are measured

more than once. For most studies in this thesis, we considered missings to be random. The best way to deal with missing at random is multiple imputation.[64] This method has been applied in **Chapters 2.3**, **2.4**, **3.1**, **3.2**, and **4.1.** Missing values in lifestyle and socioeconomic confounders were handled by multiple imputations (fully conditional specification, Markov Chain Monte Carlo method) by generating five independent datasets for all analyses, using SPSS version 17.0 for windows. In general, all possible covariates as described in the baseline characteristics, were included in the imputation procedure (these variables were imputed and used as predictor).

3.3 Internal validity

Selection bias

Selection bias may occur if the association between the determinant and the outcome is different in those who participate in the study and those who were eligible, but do not participate or are lost to follow up.[65] The Generation R Study is a population-based prospective cohort study, and its aim was to include all eligible pregnant women in a predefined area of Rotterdam. First, of all eligible children at birth, 61% participated in the Generation R Study. The percentage of women from ethnic minorities and lower socio-economic status, and of women or children with medical complications is lower among the participants than expected from the population figures in Rotterdam.[66] This selection towards a more healthy study population may probably affect the prevalence of exposure to occupational risk factors, and consequently the statistical power in our studies. Since women with lower socio-economic status participated less in our study, and these women and men are more likely to be exposed to occupational hazards, the exposure prevalence of occupational risk factors was less than expected.

This selective non-response only harms the validity of the study when the association between determinant and outcome differs between those included and those not participating in the study. This is difficult to ascertain, because we do not know the associations between determinant and outcome of those not included in the study. One can argue that selection bias will be small, because the outcome is unknown at the start of the study, but this is not always true, because the outcome under study may be associated with social, educational, and health related determinants of non-response. In many studies in this thesis, educational level was not a confounder in the association between occupational risk factors and reproductive outcomes. Two comparable pregnancy recruited birth cohorts from Scandinavia were able to compare some well-established associations between those included in the study and those not participating, and similar associations were found.[65,67] Another study by Nohr et al. showed that biased estimates in prospective cohort studies primarily arise from loss to follow up rather than from non-response at baseline.[65] In the studies in this thesis, we used information from prenatal questionnaires collected during pregnancy, and there is very little loss to follow up during pregnancy, minimising selection bias due to loss to follow up.

In almost all studies performed in this thesis, we restricted our study population to women, or men in paid employment. This approach was chosen to avoid healthy worker bias, since women in paid employment generally have better pregnancy outcomes than women without paid employment.[68] Healthy worker bias can threaten the internal validity of studies, it is the most common selection bias in epidemiological studies, and occurs because relatively healthy individuals are likely to gain employment and to stay employed.

In **Chapter 2.2** we asked women who did become pregnant about their TTP relating to their current pregnancy. The advantage of this design is that when women are asked about the current pregnancy, recall bias is minimal. The disadvantage of our design is that sterile couples are not included in the analysis and that subfecund couples are underrepresented. The inclusion of unsuccessful attempts is preferable in TTP studies; since it would ensure that estimates are not conditional on achieved conception. However, since we performed this study within Generation R, a population-based prospective cohort study on pregnant women, we were not able to include unsuccessful attempts and infertile couples in our analyses, and this may have resulted in selection bias.

Information bias

There are two main types of information bias: recall bias and misclassification. As most information in the Generation R Study was collected prospectively, recall bias is very unlikely in these studies. However, in the HAVEN study, presented in **Chapter 2.5**, information on occupational characteristics was collected at a standardised study moment of approximately 15 months after child birth. Case-control studies are more sensitive for recall bias, since data is collected retrospectively. Job characteristics were available in 99.9% of the parents, since work history in general is recalled quite easily. Recall bias in this study is very unlikely, since we did not ask for specific exposures, but only a description of the job. Moreover, the JEM used in this study ensures that exposure is classified independently from the outcome, i.e. CHD.

Misclassification can be non-differential or differential. Non-differential misclassification refers to misclassification of the outcome that does not depend on the exposure status and vice versa, while in differential misclassification this is the case. Many of the variables of interest in this thesis were obtained by self-report via postal questionnaires, and socially acceptable behaviour in answering may have occurred. For example, for the questions on physically demanding work, mothers may have overreported their exposure to these occupational risk factors. Often, exposure information in our studies was collected before assessment of the outcome, which makes differential misclassification of exposure unlikely. In addition, the examiners who collected information on foetal growth characteristics by ultrasound, and information on hypertensive disorders during pregnancy were blinded to the exposure status of the participants, which also makes differential misclassification unlikely.

Confounding

In the Generation R Study a wide range of potential confounding factors was available for analysis. Confounding will result in a spurious association between determinant and outcome. A confounding factor should be associated with both the determinant and outcome, and cannot be an intermediate in the causal pathway. The choice of which variable to include as confounder in our analyses was generally based on the following considerations. First, we tested which demographic and life style related factors were associated with our outcome of interest, and factors that significantly influenced the outcomes were considered as potential confounders. Second, all variables that were described in the literature as confounding variables, or as known determinants of the outcome, were included. Although we had information on many variables of interest, we may have missed potential confounders, resulting in residual confounding. Residual confounding due to unmeasured variables such as maternal nutrition, medication use, and physical activity during pregnancy might be possible in our studies. Thus, missing information on other adverse exposures in foetal life may have introduced residual confounding in the studies presented in this thesis.

3.4. External validity

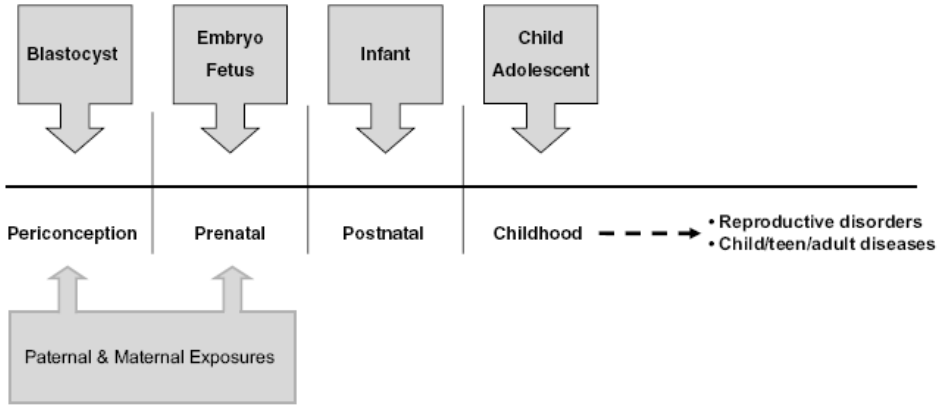
Women of lower socio-economic status and of ethnic minorities are less represented in our sample, and it is known that the lower educated jobs are often more exposed to occupational hazards. Furthermore, we restricted the study population to women, or men in paid employment. Thus, the findings of these studies may only be generalisable to the population with paid employment. The JEM used in our studies was specifically designed for the Netherlands. Since working conditions may vary over different countries, it is hard to say whether our results are also generalisable to other industrialised countries. Exposure prevalences may differ in different countries, and if the exposure prevalence is low, associations between chemicals and reproductive endpoints may not have been found. This is demonstrated by Ye et al., whereby the pregnant women in MoBa (Norway) had a higher mean concentration of urinary BPA than the Generation R (Netherlands) and the NHANES (US) women.[69] Unlike the NHANES women, the pregnant women in MoBa and Generation R were not selected to represent the whole study population nor all pregnant women in the two countries. Thus, the data do not necessarily reflect the national exposure levels in Norway or the Netherlands. It would be interesting to investigate the routes of exposure in different countries, in order to identify specific risk groups that will give insight in the generalisability of results of biomarker studies.

4. INTERPRETATION AND NEW INSIGHTS

4.1 Timing of assessment of occupational risk factors

The critical window of susceptibility is a time-sensitive interval during foetal development when exposures to environmental contaminants can disrupt or interfere with the physiology of a cell, tissue, or organ (Figure 1).[70,71]

FIGURE 1. Windows of susceptibility to environmental insults[72]



Cells have the flexibility that allows them to develop in numerous ways during the early stages of foetal development. For example, the cells in the middle mesoderm layer of the embryo have the potential to become the kidneys, skeleton, or muscle. As the cells develop more specialised characteristics, called differentiation, their flexibility decreases. If an insult, such as exposure to an environmental contaminant, occurs to the embryo prior to this differentiation, normal foetal development may still occur as other cells are able to take over for those that have been injured. However, if the insult occurs after differentiation or during times of increased cell proliferation, abnormal development can result in structural or functional defects, altered growth, and even foetal death. These times of sensitivity to environmental contaminants are referred to as critical windows of susceptibility.[73]

Unfortunately, in many studies in this thesis we were unable to investigate exposures in specific pregnancy periods. Consider morphologic development, while some structures form within one or two weeks of conception,[74] some portions of the central nervous system continue to develop throughout the entire pregnancy.[75] However, it is also known that occupational exposures, based on job title and work history, are generally continuous until the third trimester of pregnancy. In a study by Hertz-Picotto et al. variability in various exposures was described and a new measure of variability, the ratio of overall prevalence to time window specific prevalence was introduced.[76] This study shows that exposures related to location of

residence, and employment related exposures tended to be present during the bulk of the pregnancy.[76]

The occupational risk factors in most of the studies described in this thesis were obtained from the mid-pregnancy questionnaire (around 30 weeks of gestation), and the questions on starting and quitting date allowed us to check, for the majority of women, or men, whether they had worked during or before pregnancy. We carried out several sensitivity analyses to see whether women who started working before conception, differed from women who started working somewhere during the first trimester during pregnancy. In general, we observed comparable effect estimates in both analyses.

Although occupational characteristics are generally continuous over pregnancy, future studies could consider collecting trimester specific information, in order to study whether there are differences in exposures over different periods of pregnancy. The biomonitoring study measuring BPA in different trimester in pregnancy showed no differences in exposure to BPA over different trimesters of pregnancy (**Chapter 2.4**). However, in **Chapter 4.1** prenatal exposure to paracetamol and mild analgesics varied over different trimester of pregnancy, thus, insight is needed whether exposure characteristics change over the course of pregnancy and whether exposure effects differ in specific time windows during pregnancy.

4.2 Biological pathways

Chemicals acting as endocrine disruptors (EDs)

There are a number of mechanisms whereby EDs can modulate endocrine system and potentially cause adverse effects on human health. The generally accepted paradigm for receptor-mediated responses include hormone binding to its receptors at the cell surface, cytoplasm or nucleus, followed by a complex series of events that lead to changes in gene expression.[77] The main nuclear receptors involved in ED action are: estrogen receptor (ER) α and β, androgen receptor, thyroid receptor, aryl hydrocarbon receptor, and the glucocorticoid receptor. More recently, attention has been focussed on the progesterone receptor that appears to be more sensitive than ER-α and as shown in **Chapter 4.1** also a target for paracetamol. Other relevant mechanisms for EDs include inhibition of hormone synthesis, transport, or metabolism and activation of receptor through receptor phosphorylation or the release of cellular complexes necessary for hormone action. In the case of hormone synthesis, considerable research has been conducted on the aromatase inhibitors; in fact they can prevent the conversion of androgens to estrogens through a cytochrome P450 system. Several fungicides have been shown to cause aromatase inhibition as well as some widespread organotins.[78] In addition, there is growing awareness that multiple receptor systems act in concert to regulate biological functions.

The studies presented in **Chapter 2.1** and **2.2** showed adverse effects of occupational exposure to chemicals on fecundity and one of the proposed mechanisms is endocrine disruption. Endocrine disruption of spermatogenesis may be represented by four mechanisms, including

(1) epigenetic changes to the genome, (2) apoptosis of the germ cells, (3) dysregulation of androgenic signaling, and (4) disruption of Sertoli and other spermatogenesis support cells.[79] The impact of estrogens on spermatogenesis is only poorly understood. It has been shown that administration of estrogens to prostate cancer patients and to male to female transsexuals results in atrophy of the seminiferous tubules.[80,81] However, this effect might be indirect, mediated through a negative feedback on the secretion of gonadotropins. Disturbances in spermatogenesis were also observed in aromatase knockout mice,[82] this finding indicates a functional role of these hormones in relation to normal sperm production. Thus, in principle, both an excess and a lack of estrogenic action may be deleterious for spermatogenesis.

Many EDs can interact with the female reproductive system and lead to endocrine disruption in the ovary.[83] Within the reproductive system, the ovarian follicle can be considered as a very fragile micro-environment where interactions between hormones, growth factors, the oocyte and its surrounding somatic cells are essential to generate a fully competent oocyte. Disruption of this finely tuned (endocrine/paracrine) balance can lead to anovulation,[84] cystic deformation[85] or a diminished oocyte quality which jeopardises further embryo development.[86] Although originally thought to exert their effects via binding transcription factors receptors, EDs can alter endocrine function through a variety of mechanisms. Chemicals may alter the expression and/or activity of enzymes required for synthesis and/or catabolism of ovarian sex steroids, and may alter the expression of hormone receptors and/or their ability to bind their endogenous ligand. More studies, however, are needed to further understand the mechanisms of action of currently known EDs, identify and characterise new EDs, and expand toxicological research beyond commonly studied receptors and pathways. Although in vitro experiments suggest a role for EDs in disturbing the tightly regulated endocrine and paracrine signaling in the different cells of the ovarian follicle,[87-89] these exposure experiments can only be related to the in vivo situation if environmental relevant ED concentrations are considered. Information on the contaminant status of female follicular fluid is indispensable, which implies the need for continuous monitoring of EDs in follicular fluid.[90-93] An example of which way forward is a recent study by Petro et al. which showed that ED contamination in the follicular micro environment was linked to fertilisation success rate and the chance of development of oocytes into high quality embryos.[94]

Hormones play a vital role in a complex series of events, during which the single cell of the fertilised egg forms into the millions of cells that make up the newborn. Any disruption in maternal or foetal hormone levels has the potential to negatively affect foetal development. The EDs most commonly associated with reproductive anomalies are the xenoestrogens such as bisphenol A (BPA), polychlorinated biphenyls (PCBs), and antiandrogens such as phthalates. BPA's toxicity and reproductive dysfunction has been linked to BPA's binding of the estrogen receptor as well as nuclear-receptor independent activation of key cellular signaling system.[95] BPA can target the placenta directly.[96] Mouse cytotrophoblast cells cultured in physiologic doses of BPA demonstrate abnormal labyrinthine development and increased rates of apoptosis.[97]

In addition, BPA decreases placental aromatase activity leading to lower levels of estrogen production and decreased the amount of estrogen and progesterone receptor expression in the placenta.[98,99] These underlying mechanisms possibly play a role in the observed effects of BPA on intrauterine growth (**Chapter 2.4**). Further research could focus on measuring BPA in amniotic fluid to see whether these concentrations are related to adverse foetal development and placenta function, or possibly the determination of receptor expression in the placenta when collected after birth.

Alternative routes for toxic effects of chemicals on reproduction

Various pathways have been described how occupational and environmental chemicals may affect human reproduction. Direct toxicological effects have been described for example for exposure to lead. In **Chapter 2.2** an association was found between paternal occupational exposure to heavy metals and prolonged TTP, suggesting adverse effect of these substances on spermatogenesis. For lead exposure, mechanistic studies have suggested that lead exposure disrupts all levels of the reproductive axis.[15] Follow up clinical studies are less definitive than animal studies, but support the evidence that the toxicity occurs at all levels of the reproductive axis, with some studies concluding that the primary site of toxicity is the central nervous system, with other concluding that the gonad is the most sensitive organ.[15,100,101] Recent evidence suggests that lead interferes with the ability of spermatozoa to undergo the acrosome reaction, thus leading to infertility.[102] Genetic variability in response to lead exposure is suggested by the finding that ion channel polymorphisms may cause differential sensitivities to lead exposure, both in the animal model and clinical studies.[103]

Furthermore, there is increasing evidence that induction of oxidative stress may affect fecundity and also foetal development. An increase in oxidative stress can been seen in ≤80% of clinically proven infertile men, and exposure to environmental toxicants is a major factor contributing to such an increase.[104-106] In a recent review, the disruptive effects of environmental toxicants on cell junctions mediated by non-receptor tyrosine kinases and cytokines through oxidative stress are highlighted, because such damage is often observed in low level exposure before apoptosis occurs.[107] It is recognised that the foetus is vulnerable to the minutest concentration of toxic chemicals as compared to adults. This may be due to the fact that the foetus is growing at a rapid rate and is immature in a number of functional aspects. In **Chapter 2.3** and **2.4** we described that chemical exposure adversely affects intrauterine growth and placental weight. Teratogenicity via bioactivation and direct formation of reactive oxygen species by a number of xenobiotics, and the level of oxygen in the intrauterine environment plays a critical role in pregnancy by affecting embryo development and placentation. Oxidative stress is thought to alter cellular function potentially resulting in in utero death or teratogenicity.[108] Embryonic processes regulating the balance of reactive oxygen species formation, oxidative DNA damage and repair, and oxygen species mediated signal transduction may be important determinants of teratological risk.[109]

The findings in **Chapter 2.5** on paternal occupational exposure to chemicals, that may act as EDs, on the occurrence of CHDs are possibly linked to the effects of these chemicals on spermatogenesis. Environmental pollutants have been linked with epigenetic variations, including induced changes in DNA methylation, histone modifications and microRNAs.[110-113] Dynamic chromatin remodeling is required for the initial steps in gene transcription, which can be achieved by altering the accessibility of gene promotors and regulatory regions.[114] Epigenetic factors, including DNA methylation, histone modification, and microRNAs participate in these regulatory processes.[115,116] Changes in these epigenetic factors have been shown to be induced by exposure to environmental pollutants and some of them were linked with different diseases.[117-119] Chemicals might disturb the epigenetic programming during maturation of the sperm cells, which may result in derangements in imprinted genes in particular in embryonic tissue, which may subsequently lead to birth defects.[120-122] Furthermore, alterations in these epigenetic processes by exposure to chemicals during early pregnancy may also results in altered foetal development, for example decreased foetal growth. Studies investigating the effects of chemicals on these epigenetic processes are urgently needed, for example studies comparing DNA methylation in exposed and unexposed workers during pregnancy. And if possible, link these effects to adverse effects on foetal development and pregnancy outcome.

Endocrine disruption or not, that's the question

It is clear that the endocrine system presents a number of target sites for the induction of adverse effects by environmental agents. There are numerous examples demonstrating that reproductive and developmental processes may be exquisitely sensitive to exposure and there are effects induced by presumed EDs in a variety of species.[123] Although animal studies have raised concern on the influence of EDs on reproduction, exposure levels far above those found in humans have been needed to evoke reproductive toxicity in the animal models. Human data are inconclusive and have raised the question of whether EDs can have any impact on hormonal function and thus health consequences when natural hormones are present. Indeed, many contaminants with hormone-like activity are much less potent than endogenous hormones themselves;[124] 17-β-estradiol was for instance estimated to be 17000 times more potent than p,p'-dichlorodiphenyltrichloroethane. However, humans are exposed to a multitude of agents, and when present in sufficient number and/or concentration, they might in principle act together to impact on the actions of endogenous hormones. Whether such impacts will be physiologically relevant is still not known, but in a worst case scenario, there are no threshold levels below which there are no effects at all. Not only is there a need for better test procedures (both in vivo and in vitro) to characterise the potential of EDs to disrupt endocrine function, but there is also a need for more information on the transport, fate and bioavailability of chemicals released into the environment and exposures occurring through certain occupations.

The results of the studies on chemical exposure in both general populations in this thesis may be possibly linked to endocrine disruptive effects of these chemicals. However, the findings cannot be used to exclude the possibility of other biological mechanisms.

Physically demanding work and foetal growth

We found effects of long periods of standing on foetal head circumference, and effects of long working hours on both foetal weight and foetal head circumference (**Chapter 3.2**). Several mechanisms have been suggested to explain the possible adverse influence of physically demanding work during pregnancy on the foetus. Work involves muscle action, causing an increase of sympathetic vasomotor activity in working muscles proportional to the severity of work. Hence, when cardiac output increases rapidly during muscular activity, most blood goes to the working muscles and proportionally less arrives in the other viscera, which during pregnancy, includes the placental bed. Thus, heavy physical work is thought to reduce the blood flow to the uterus and placenta, thereby reducing the availability of oxygen and nutrients for the foetus.[125-127] Physically demanding work has also been described in relation to an increased release of catecholemines. An increased release of catecholemines, with resultant arteriolar constriction, has been hypothesised to play a role in the aetiology of adverse pregnancy complications.[128] It would be interesting to see whether physically demanding work influences early placentation, by measuring the effects of physically demanding work on uteroplacental blood flow and resistance, and whether it influences catecholamine levels.

Paracetamol and reproductive disorders

In **Chapter 4.1** we found an association of mild analgesics use during the second trimester of pregnancy with an increased occurrence of cryptorchidism in the offspring. Our findings corroborate findings from earlier epidemiological studies and the underlying biological mechanisms for this trimester specific association has been described previously. Experimental rat models have shown that normal androgen action during a critical male programming window (gestational day 15.5-17.5), which is though to correspond to the second trimester of pregnancy in humans, is crucial for the programming of the testis descent.[52] Acetaminophen (paracetamol) possesses highly selective analgesic and antipyretic effects that results from its inhibitory actions on the synthesis of prostaglandins. Prostaglandins are generated by the oxygenation of arachidonic acid to the unstable intermediate prostaglandin H2 by prostaglandin H2 synthase, of which there are two major isoforms, also commonly referred to as cyclooxigenase (COX) 1 and 2, respectively.[129] Acetaminophen is an inhibitor of both COX enzymes in purified enzyme preparations.[130]

The testosterone dependant differentiation of the male reproductive tract requires the continuing synthesis of prostaglandins.[131] Suppression of prostaglandin production by interfering with the arachidonic acid cascade at the level of release of arachidonic acid from cell membrane lipids or by inhibiting COX enzymes diminishes foetal androgen action and compromises male

sexual differentiation.[132] Accordingly, Kristensen et al. demonstrated that acetaminophen is capable of suppressing foetal androgen synthesis in male rats exposed ex vivo to the drug during late foetal life. The day before birth the male foetuses showed reduced anogenital distances, another sign of diminished androgen action.[51] In a subsequent paper, Kristensen et al. were able to pinpoint the suppression of prostaglandin synthesis by acetaminophen and other NSAIDs to the inhibition of COX enzymes.[133] Importantly, the same authors revealed many other putative EDs, including phthalates and other phenolic agents, possess prostaglandin-inhibitory potential. In the light of these observations, it appears biologically plausible that not only NSAIDs, but also other EDs may contribute to increasing the risks of developing cryptorchidism by interfering with prostaglandin synthesis. It becomes necessary to address this possibility in further epidemiological studies.

4.3 Exposure assessment strategies

Timing of exposure assessment during pregnancy has already been described as an important issue in exposure assessment strategies. Another important issue is accurate and valid occupational exposure data, one of the most serious weaknesses in the studies on occupational hazards.[134]

The importance of reliable and valid methods to measure occupational exposures in general population studies has become a central focus of research efforts over the past decade.[135,136] Traditionally, studies have been based on the collection of information on job title as a surrogate for occupational exposures. In the early 1980s, the Job-Exposure-Matrix (JEM) method was proposed to translate information on job title into specific exposures. In **Chapter 2.2, 2.3** and **3.1** we used an extended version of the JEM by Van Tongeren et al.[137] The validity of JEMs, however, has been shown to vary greatly from study to study and JEMs are unable to account for variability in exposures within occupations.[138] Additional difficulties may arise when a JEM is applied to populations different from that originally targeted.[139] Studies performed by Kennedy et al. and Semple et al. indicate that the general problem of JEM exposure misclassification can be partly resolved by refining JEM exposure estimates with tasks specific information from questionnaires or interviews.[140,141] In Generation R this information on work tasks was available from the questionnaire and used to correctly apply the JEM. The new updated JEM incorporated more knowledge on chemicals with EDs, and was made more specific by assigning exposure probability scores for chemical subcategories. As exposure prevalences in the general population are usually low and most pollutants in the workplace are associated with moderate or low risks of adverse outcomes, improvement in assessing exposure is crucial to design informative epidemiological studies. Results of epidemiological studies should be interpreted in the light of the quality of the exposure assessment methods used, and if available, information on the validity of the assessment procedure should be included in the scientific report. The way forward, would be to validate the JEM through occupational hygiene samples or measurements

in biological media. These studies are currently planned within the Generation R Study, and will provide insight in the performance of the JEM.

When interpreting the results of epidemiological studies using biological measurements, it must also be stressed that exposure measurements are also prone to error. For measurements in urine day to day variation is particularly critical for metabolites with a short elimination half life. Biomonitoring is normally not feasible for biomarkers with a half life less than two hours. For the monitoring of chemicals that have long half lives, it is generally agreed that biomarkers of exposure have considerable advantages due to stability and require relatively few measurements to define exposure. Rather than being a complication, the variation between and within individuals is valuable in determination of the risk. In **Chapter 2.4** we studied BPA concentrations in prenatal urine in three trimesters of pregnancy. This study gave insight in the effects of BPA on foetal growth and we were able to evaluate the influence of the measurement strategy chosen on the observed effect estimates. This study raises important questions on how many subjects must be measured and how many times they need to be measured. The BPA-foetal growth relation may fit the profile of a setting where, for a fixed total number of measurements, more replicates and fewer subjects maximises power.[31] The review in **Chapter 2.1** also showed that smaller studies with a detailed and accurate exposure assessment more often showed significant associations with prolonged time to pregnancy, emphasising the need for detailed exposure assessment.

An important objective for future direction of biomarker studies is to study the determinants of exposure to certain chemicals. Activities or circumstances that can influence environmental exposures can include: food consumption, drinking water sources, products used, work, home environment, agricultural or industrial activities, energy production, transportation, and waste disposal activities. Also certain demographic, as well as life style related factors may be related to the level of exposure to chemicals. In addition, some risk groups may be genetically more susceptible to certain exposures of health concerns than other due to human genetic variability, or specific genetic polymorphisms, For example, susceptibility of some individuals to asthma and asthma triggers such as air pollution is well understood to have a genetic component.[142] Another example involves differences in leukemia risk associated with prenatal pesticide exposure. Children with leukemia were shown to carry specific genetic characteristics that altered the ability of their liver to metabolise foreign substances, including pesticides.[143] The critical issues for the use of biomarkers in occupational health research in the future will be:

– The extent to which biomarkers have been validated. The further development of standardised methods, which can be properly evaluated and validated, especially in inter-laboratory trials, is very important. Mass spectrometry is a universal method of detection and therefore it is considered important to develop this further for biomonitoring and biomarker studies. The development of toxicogenomics, proteomics, and metabolomics is also viewed as very important, especially for studies on mechanistic aspects. The

Contamed project is currently working on the development of such methods. By using metabolomic profiling and a combination between analytical chemistry with in vitro ED mode-of-action screens bioassay-directed fractionations, previously unrecognised EDs as well as endogenous biomarker metabolites can be identified. Biomarkers will be developed to determine internal ED load, using bioassays, to prepare ground for epidemiological studies.

- More research is needed to link biomonitoring data quantitatively to health risks.[144] The mere presence of chemicals in a biological specimen does not equal risk, and the ability to measure chemicals is far outpacing the ability to interpret its meaning.[145]
- It is important to establish whether there are levels of exposure with no observable effects.

4.4 Population-based studies

A limitation of a population-based approach in studying occupational risk factors is lack of power to identify the specific role of occupational exposure with a low prevalence. For example, in the study of maternal occupational risk factors for hypertensive pregnancy complications (**Chapter 3.1**), we were unable to demonstrate a negative effect of occupational risk factors on hypertensive complications during pregnancy.

An advantage of population-based studies in occupational epidemiology is that they give information on the public health impact of occupational risk factors, and thus the impact on population level. This information may be used to guide the need for preconception counseling of parent to be. Due to the fact that the prevalence of occupational exposure to chemicals of fathers to be is generally higher than the exposure of mothers to be, overall 15.9% versus 6.2%, the population impact will be higher for fathers. Overall, we may conclude that population impact of occupational exposure to chemicals, and physically demanding work is low. Two explanations may be sought, as mentioned earlier the low exposure prevalences, but also the moderate effects of these occupational risk factors, with Odds Ratios (ORs) ranging between 1.5 and 2.5. For exposure to mild analgesics and bisphenol A, the exposure prevalence is much higher compared to occupational chemical exposure. The population attributable fraction (PAF) for the association between use of mild analgesics during the second trimester and reproductive disorders in the offspring is relatively large, 24%. Thus, if causality could be established, mild analgesics explain 24% of 1% (prevalence cryptorchidism), we need to inform approximately 400 women not to take paracetamol during the second trimester of pregnancy to prevent one case or cryptorchidism.

Although the population impact is small, the effects of maternal occupational exposure to chemicals and physically demanding work on foetal growth seem of similar magnitude than other well-known life style factors (**Chapter 2.3** and **Chapter 3.2**). The effects of certain chemicals on foetal growth resulted in a possible difference of approximately 100-400 gram difference in birth weight, and long working hours in approximately 150-200 gram difference in birth weight. These differences are comparable with other life style factors, and one could

argue that women in specific occupations should be informed about their risks. The effects of BPA on foetal growth were even larger, with an estimated difference of 683 grams in birth weight and 3.9 cm in head circumference at birth. These results need to be confirmed in future research, and insight in routes of exposure is critical in order to define targets for prevention.

Since working conditions are a modifiable factor, preconception counseling presents a strategy to minimise the environmental and occupational sources of reproductive risks facing the preconception person. Occupations in which women have a high exposure probability are agricultural and horticultural workers (pesticide exposure), hairdressers, beauticians, furniture makers (phthalate exposure), cleaners (alkylphenolic compounds), nurses, child care givers, saleswomen (lifting heavy loads), and stewardesses, physicians, nurses (night shifts). Since the effects for chemicals and physically demanding work are considerable, one could argue that pregnant women, for example women working in agriculture, must be informed about the risks of pesticide exposure in the workplace. However, the underlying mechanisms for chemical exposure and physically demanding work and adverse foetal development are still largely unclear, and results from earlier studies conflicting, warranting further research into this important topic.

Integrating all evidence presented in this thesis, we can conclude that the effects of physically demanding work on pregnancy are moderate, but the prevalence of these occupational risk factors is considerable. This is in contrast to exposure to chemicals, where the prevalence is very low, but the effects on pregnancy are considerable. Approximately 30% of pregnant women take mild analgesics during pregnancy, and mild analgesics seem to increase the risk of cryptorchidism, thus, discouraging pregnant women to take mild analgesics seems justified.

5. RECOMMENDATIONS

The effects of paid employment during pregnancy on foetal and maternal health are topical subjects. Anyone searching for background information needed in patient counselling may be overwhelmed by the large and sometimes contradictory body of research compiled in the past decade. In light of the limitations of existing data, the biomedical literature does not provide consistent evidence for the presence or absence of risk for many contaminants. Overall, the strongest evidence of environmental contaminant exposures interfering with healthy reproductive function in adult females is for heavy metals, particularly lead. Compounds that can influence the normal balance of hormones, including many pesticides and BPA, also appear related to adverse reproductive outcomes.

5.1 Recommendations for future research

In my opinion, there are several promising prospects for future research in the field of occupational epidemiology.

1. Exposure characterisation

Development of better exposure characterisation, most notably the development of biomarkers, is of paramount importance. New biomarkers for exposure need to be developed and validated in order to contribute to prevention of occupational related diseases. Biologic measurement collection and biobanking should be incorporated into epidemiologic study designs in order to facilitate future research.

2. Validation of the Job-Exposure-Matrix

The JEM used in several of the studies presented in this thesis needs to be validated (**Chapter 2.2, 2.3, 2.5**). Since the JEM provides a rather crude measure of exposure, and no information is available on occupational hygiene measurements from companies, or biomonitoring of workers, research in this field is urgently needed. For example, with use of biomonitoring, concentrations in several occupations can be compared to the concentrations of exposure in the general population. These studies will be a step forward in better understanding the risks of occupational exposures, and give insight in exposure levels of chemicals of occupationally exposed workers.

3. Endocrine disruptors, and other biological pathways

In **Chapter 2.2, 2.3, 2.4** and **2.5** we described the effect of chemicals on various aspects of reproduction, and a possible underlying mechanism for effect is endocrine disruption. To confirm this assumption, mechanistic research into the effects of EDs such as pesticides, phthalates, flame retardants, and perfluorinated acids on reproduction is needed. Is it possible to demonstrate the endocrine disruptive effects of chemicals on sperm cells, or oocytes? How are the endocrine system and markers of healthy reproductive function influenced by the complex mixtures of environmental toxicants routinely encountered by men and women. Possibly by exposing laboratory animals to EDs and measuring distortions in endocrine parameters. In human cohort studies, occupationally exposed individuals may be tested for endocrine distortions, by measuring the effects of chemical exposure on levels of LH, FSH, testosterone, estradiol, and other hormones, such as thyroid function. The effects of EDs on foetal growth and development may be explored in more detail in animal models, exposing animals to relevant concentrations of chemicals, analysing receptor functions in the placenta, or other tissue, measuring EDs in amniotic fluids. In pregnancy based cohort studies, the effects of chemicals measured in human body fluids on placental function and foetal development can be explored more extensively.

Furthermore, genetic studies are needed to identify populations that are genetically susceptible for exposure to chemicals. Studies may focus on genetic material from exposed and unexposed women or men, in order to see whether there are differences in epigenetic profiles and other genetic markers.

4. Longitudinal study designs

Wherever possible, research efforts should be coordinated across the life stage using longitudinal study designs. To maximise efficiencies, researchers should cooperate with each other and pool data across studies and follow up existing cohort datasets, thus allowing investigators to assess offspring health and later life health events for original cohort members.

5.2 Recommendations for clinical practice

The findings in our studies on occupational and environmental risk factors do not present a strong foundation for preventive measures yet. From a clinical perspective, important modifiable risks appear to be associated with exposures specific to unique populations or occupational groups (for example pesticide applicators). Questions about such exposures may not come up in a typical patient history, but clinicians should consider them during pre-pregnancy counseling or if patients encounter reproductive difficulties. As part of promoting a healthy lifestyle, physicians can comment on hobbies and occupational exposures as well as encouraging patients to avoid unnecessary exposures. Where hazards are known or suspected, for example with applying pesticides, women should take any recommended precautions and follow label instructions.

From a public health perspective, we should remember that reproductive health is couple dependent, and is an accumulation of a lifetime of experiences and exposure scenarios. The interdependence of reproductive health endpoints calls for better integration of longitudinal studies with multiple endpoints.

6. GENERAL CONCLUSION

This thesis demonstrates that exposure to some chemicals at work as well as physically demanding work have varying influence on all aspects of reproductive health, starting with a delayed time to pregnancy, a less prolific growth of the foetus, and birth defects such as congenital heart defects and cryptorchidism. The use of mild analgesics during pregnancy also increases the risk of cryptorchidism in the male offspring. Thus, work-related and environmental risk factors may influence reproduction and already have their effects on early development of human life. An important proposed underlying mechanism is endocrine disruption, which influence can be profound because of the crucial role hormones play in controlling reproduction and development. Although the prevalence of occupational exposure to chemicals is relatively low, the effects on foetal growth and fecundity are considerable, and one could argue that workers in specific occupations must be informed about the risks of chemicals in the workplace. Since approximately 30% of pregnant women use mild analgesics during pregnancy, and several studies showed an increased risk with reproductive disorders, it seems justified to inform women about their risks. Further studies are needed to confirm our findings and to explore

the underlying mechanisms. If the results of future studies point in the same direction, efforts will be needed to reduce the exposure to occupational risk factors during pregnancy and to increase awareness among pregnant women.

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PART 6

SUMMARY / SAMENVATTING

SUMMARY

This thesis demonstrates that exposure to some chemicals at work as well as physically demanding work have varying influence on all aspects of reproductive health, starting with a delayed time to pregnancy, a less prolific growth of the foetus, and birth defects such as congenital heart defects and cryptorchidism. The use of mild analgesics during pregnancy increases the risk of cryptorchidism in the male offspring. Thus, work-related and environmental risk factors may influence reproduction and already have their effects on early development of human life.

For several work-related and environmental risk factors associations with reproductive effects have been established and translated into legislation, such as mandatory provisions for pregnant women preparing antineoplastic drugs or being exposed to lead. With the increasing labour force participation among women in Western countries, many women will work during their reproductive years. This will increase the likelihood that women during their reproductive years will be exposed to a variety of risk factors at work that may affect their reproductive abilities and the outcome of their pregnancy, such as spontaneous abortion, hypertensive disorders, intrauterine growth restriction, and adverse birth outcomes. Occupational exposures may also interact with foetal development, resulting in health effects in the offspring, such as congenital malformations and neurobehavioural disorders at young age. However, for many other work-related and environmental risk factors, the scientific evidence is less consistent. Furthermore, little is known about the underlying mechanisms through which work-related risk factors, such as exposure to chemicals, and physically demanding work, affect foetal development.

In **Part 1**, the main objectives of this thesis were described: (1) to examine the effects of exposure to chemicals and (2) physically demanding work on various domains of reproduction, and (3) to study the relation between exposure to endocrine disruptors (EDs) and congenital anomalies, including reproductive tract abnormalities. To address these aims, we have evaluated the effects of exposure to chemicals on fecundity (time to pregnancy), intrauterine growth, hypertensive disorders and birth outcomes. We evaluated the effects of physically demanding work on intrauterine growth, hypertensive disorders, and birth outcomes. Furthermore, exposure to EDs (including paracetamol) in relation to congenital heart defects (CHDs), cryptorchidism and hypospadia was investigated.

Most studies were embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands. In total, 8880 pregnant women with a delivery date between April 2002 and January 2006 were enrolled during pregnancy. Extensive assessments were carried out during the first trimester (gestational age < 18 weeks), second trimester (gestational age 18-25 weeks) and third trimester (gestational age >25 weeks), including physical examinations, questionnaires, interviews, and biological samples. Information on occupational and environmental risk factors was mainly derived from prenatal questionnaires. Information on possible confounders and pregnancy characteristics were obtained from questionnaires, physical examinations, ultrasound examinations, biological

samples and medical records. One study within this thesis was embedded in the HAVEN study, a case-control-family study, designed to investigate determinants in the pathogenesis of CHDs. Recruitment of case and control children took part between June 2003 and January 2010, for case children four university medical centres enrolled the children and parents, and control children and parents were enrolled in collaboration with child health care centres. Children with CHD diagnosed by paediatric cardiologists in the first 15 months after birth were enrolled. The information on occupational and environmental risk factors was obtained from a questionnaire, which was filled out by the mother and father separately.

Part 2 presents different studies that examined the associations between exposure to chemicals before or during pregnancy on various reproductive endpoints. In **Chapter 2.1** we systematically reviewed the literature on occupational exposure to chemicals and time to pregnancy (TTP). For lead, strong indications for adverse effects on TTP were present, supporting the mandatory provisions for pregnant women being exposed to lead in many countries. These indications were also found for pesticide exposure, and one could argue that couples working in agriculture or horticultural trades must be informed about the risks of pesticide exposure. Epidemiologic evidence on other chemicals, such as organic solvents, and other metals remains equivocal, hampering clear counselling of couples who are trying to become pregnant. In **Chapter 2.2** we hypothesised that occupational exposure to chemicals that may act as EDs may lead to a prolonged TTP. We observed that paternal occupational exposure to heavy metals and overall exposure to EDs was significantly associated with a prolonged TTP. For maternal occupational exposure to EDs we also observed decreased fecundity, however, due to the low prevalence of maternal occupational exposure to EDs these associations were not statistically significant. Thus, we provided indications for adverse effects of occupational exposure to EDs on TTP. **Chapter 2.3** showed that maternal occupational exposure to chemicals, possibly acting as EDs, influenced various domains of foetal growth. Exposure to polycyclic aromatic hydrocarbons was associated with lower growth rates for foetal weight, exposure to phthalates with lower growth rates for both foetal weight and foetal length, exposure to alkylphenolic compounds with lower growth rates for foetal head circumference, and exposure to pesticides with lower growth rates for foetal length. Furthermore, we were able to demonstrate that exposure to pesticides and phthalates was associated with a decreased placental weight. We provide some evidence that exposure to chemicals may lead to suboptimal placental development and subsequently to decreased foetal growth. In **Chapter 2.4** high exposure to bisphenol A (BPA) was associated with impaired foetal growth. Compared with women with low concentrations of BPA in urine, women with high concentrations showed lower growth rates for foetal weight and head circumference. Furthermore, we were able to evaluate the measurement strategy chosen on the observed effect estimates. The BPA-foetal growth relation may fit the profile of a setting where, for a fixed total number of measurements, more replicates and fewer subjects maximises power. This study showed the need for better exposure assessment strategies, and may explain why some studies report negative findings. In **Chapter 2.5** we hypothesised that parental occupational to chemicals might influence the occurrence of

CHDs. Paternal occupational exposure to phthalates was associated with a higher incidence of CHDs in general, and several chemicals, including phthalates, polychlorinated compounds and alkyphenolic compounds were associated with specific CHD phenotypes. Distortion of epigenetic mechanisms by chemicals might be underlying the effect of paternal occupational exposure to chemicals on the occurrence of CHDs.

In **Part 3** we present two studies on the relation between physically demanding work and pregnancy. In **Chapter 3.1** the effects of several occupational risk factors, including physically demanding work, working hours and chemical exposure did not seem to influence the occurrence of hypertensive disorders during pregnancy. These findings do not indicate an effect of these occupational risk factors on maternal cardiovascular health. **Chapter 3.2** showed that physically demanding work, specifically long periods of standing, and long working hours, influenced the growth rates for foetal weight and head circumference. A negative effect of long periods of standing on growth rates for foetal head circumferences was found. For long working hours, effects on both foetal weight and head circumference were found. These findings indicate that physically demanding work during pregnancy, and long working hours during pregnancy, may affect intrauterine growth. However, we did not find consistent associations between these risk factors and adverse birth outcomes.

Part 4 of this thesis focussed on use of mild analgesics during different periods in pregnancy and the risk of cryptorchidism and hypospadia in the offspring. In **Chapter 4.1** we showed that use of mild analgesics during the second trimester of pregnancy was associated with an increased risk of cryptorchidism in the offspring. Since a relatively high proportion of pregnant women is using paracetamol during pregnancy, population impact may be substantially. The population attributable fraction was calculated for second trimester use, and indicated that if causality could be established, approximately 24% of the cases of cryptorchidism could be attributed to the use of mild analgesics. Paracetamol, and other mild analgesics inhibit the production of androgens in the developing foetus, impairing the androgen dependent descent of the testis.

Finally, **Part 5** summarises the main findings of the studies in this thesis and discusses the methodological considerations and interpretation of the findings. Furthermore, suggestions for future research are proposed.

In conclusion, the studies described in this thesis demonstrate that various occupational and environmental risk factors may adversely influence various domains of human reproduction, including fecundity, intrauterine growth, hypertensive disorders during pregnancy, and congenital malformations. Further studies are needed to corroborate or refute these findings and to elucidate the underlying mechanisms. Results of future studies and the results of the current studies may increase the awareness of the potential harmfull effects of certain occupational and environmental exposures in general, and in particular in pregnant women and their unborn child.

SAMENVATTING

Dit proefschrift toont aan dat blootstelling aan bepaalde chemische stoffen op het werk, alsmede zwaar fysiek werk, van invloed kan zijn op verschillende aspecten van de voortplanting, waaronder een verlengde duur tot zwangerschap, een verminderde groei van de foetus, en aangeboren afwijkingen zoals aangeboren hartafwijkingen en cryptorchisme. Daarnaast beschreven we dat het gebruik van pijnstillers tijdens de zwangerschap het risico op cryptorchisme verhoogt. Aan het werk gerelateerde en omgevingsgerelateerde risicofactoren kunnen de voortplanting beïnvloeden en deze effecten kunnen al optreden tijdens de vroege ontwikkeling van de mens.

Voor verschillende werk gerelateerde en omgevingsgerelateerde risicofactoren zijn er effecten beschreven op de voortplanting en dit heeft zich vertaald in wetgeving, zoals voor zwangeren die antineoplastische medicijnen klaarmaken of zwangeren die blootgesteld zijn aan lood. Door het nog steeds toenemend aantal vrouwen op de arbeidsmarkt in Westerse landen, zullen veel vrouwen werken tijdens de vruchtbare jaren. Dit verhoogt de kans dat vrouwen tijdens de vruchtbare jaren worden blootgesteld aan verscheidene risicofactoren op het werk die de voortplantings capaciteiten en de uitkomst van de zwangerschap, zoals spontane miskraam, hoge bloeddruk tijdens de zwangerschap, foetale groeipatronen, en ongunstige geboorteuitkomsten, zouden kunnen beïnvloeden. Beroepsmatige blootstellingen kunnen de foetale ontwikkeling beïnvloeden, en dat zou kunnen resulteren in ongunstige gezondheidsuitkomsten in het nageslacht, zoals aangeboren afwijkingen en gedragsneurologische afwijkingen op jonge leeftijd. Voor veel andere werk gerelateerde en omgevingsgerelateerde risicofactoren is het wetenschappelijk bewijs minder consistent. Verder is maar weinig bekend over het onderliggende mechanisme hoe werk gerelateerde risicofactoren, zoals blootstelling aan chemische stoffen, en zwaar fysiek werk, de foetale ontwikkeling kunnen beïnvloeden.

In **Deel 1**, beschreven we de belangrijkste doelstellingen van dit proefschrift: (1) het onderzoeken van de effecten van blootstelling aan chemische stoffen en (2) zwaar fysiek werk op verschillende domeinen van de voortplanting, en (3) het onderzoeken van de relatie tussen het gebruik van pijnstillers tijdens de zwangerschap en aangeboren afwijkingen, zoals afwijkingen aan de voortplantingsorganen bij jongens. Om deze doelstellingen te kunnen onderzoeken, hebben we de effecten van blootstelling aan chemische stoffen op de duur tot zwangerschap, foetale groeipatronen, hoge bloeddruk tijdens de zwangerschap en ongunstige geboorteuitkomsten onderzocht. We evalueerden de effecten van zwaar fysiek werk op de foetale groeipatronen, hoge bloeddruk en ongunstige geboorteuitkomsten. Verder hebben we de relatie tussen stoffen die het hormoonstelsel beïnvloeden, zoals pijnstillers, en de relatie met aangeboren hartafwijkingen, cryptorchisme en hypospadie onderzocht.

De meeste studies in dit proefschrift werden uitgevoerd binnen het Generation R onderzoek, een populatie-gebaseerde prospectieve cohort studie vanaf de vroege zwangerschap in Rotterdam, Nederland. In totaal namen 8880 zwangere vrouwen met een bevallingsdatum

tussen april 2002 en januari 2006 deel aan het onderzoek. Uitgebreide meetmomenten vonden plaats tijdens het eerste trimester (zwangerschapsduur < 18 weken), tweede trimester (zwangerschapsduur 18-25 weken) en derde trimester (zwangerschapsduur > 25 weken) van de zwangerschap en omvatte lichamelijk onderzoek, vragenlijsten, interviews, en verzameling van lichaamsmateriaal. Informatie over werk gerelateerde en omgevingsgerelateerde risicofactoren werd verkregen uit prenatale vragenlijsten, zo ook informatie over mogelijk verstorende variabelen, en zwangerschaps kenmerken, lichamelijk onderzoek, echo onderzoek, lichaamsmateriaal en medische dossiers. Een van de onderzoeken in dit proefschrift werd uitgevoerd binnen de HAVEN studie, een case-control familie studie, die de determinanten in de pathogenese van aangeboren hartafwijkingen onderzoekt. Werving van case en controle kinderen vond plaats tussen juni 2003 en januari 2010, case kinderen en hun ouders werden gevraagd om deel te nemen via vier universitaire ziekenhuizen, en controle kinderen en hun ouders werden gevraagd om deel te nemen via de consultatiebureaus. Kinderen met een aangeboren hartafwijking gediagnosticeerd door een kindercardioloog in de eerste vijftien maanden na de geboorte werden gevraagd om deel te nemen. De informatie over werk gerelateerde en omgevingsgerelateerde risicofactoren werd verkregen uit een vragenlijst, die door de moeder en vader apart werd ingevuld.

Deel 2 beschrijft verschillende studies die de relatie tussen blootstelling aan chemische stoffen voor of tijdens de zwangerschap en voortplantingsparameters onderzochten. In **Hoofdstuk 2.1** hebben we systematisch gezocht in de literatuur naar artikelen over beroepsmatige blootstelling aan chemische stoffen en duur tot zwangerschap en dit samengevat in een review artikel. Voor blootstelling aan lood, bestaan sterke aanwijzingen dat dit de duur tot zwangerschap negatief beïnvloedt. Dit ondersteunt de genomen voorzorgsmaatregelingen voor zwangere vrouwen die zijn blootgesteld aan lood in verschillende landen. Verder, zijn deze aanwijzingen ook gevonden voor blootstelling aan pesticiden, en men zou kunnen beargumenteren dat koppels die werken in agrarische beroepen geinformeerd moeten worden over de risico's van blootstelling aan pesticiden. Epidemiologisch bewijs voor de negatieve effecten van andere chemische stoffen, zoals oplosmiddelen, en metalen, blijft tweestrijdig, wat counseling van koppels die proberen om zwanger te worden bemoeilijkt. In **Hoofdstuk 2.2** onderzochten we de hypothese dat beroepsmatige blootstelling aan chemische stoffen, die het hormoon stelsel kunnen beïnvloeden, zou kunnen leiden tot een verlengde duur tot zwangerschap. Blootstelling van de vader via het beroep aan metalen en blootstelling aan chemische stoffen in het algemeen was significant geassocieerd met een verlengde duur tot zwangerschap. Voor moeders observeerden we dat blootstelling aan chemische stoffen via het beroep in het algemeen leidde tot een langere duur tot zwangerschap, maar door de lage prevalentie van blootstelling onder moeders waren deze associaties niet significant. Samenvattend, we hebben indicaties gevonden voor negatieve effecten van beroepsmatige blootstelling aan hormoonverstorende stoffen op duur tot zwangerschap. **Hoofdstuk 2.3** laat zien dat beroepsmatige blootstelling van de moeder aan chemische stoffen tijdens de zwangerschap verschillende domeinen van

foetale groei beïnvloedt. Blootstelling aan polycyclische aromatische koolwatervloeistoffen was geassocieerd met lagere foetale groeisnelheden voor het foetale gewicht, blootstelling aan weekmakers (ftalaten) met lagere groeisnelheden voor zowel foetaal gewicht als foetale lengte, blootstelling aan alkylerende stoffen met lagere groeisnelheden voor foetale hoofdomtrek en blootstelling aan pesticiden met een lagere groeisnelheid voor foetale lengte. Verder toonden we aan dat blootstelling aan pesticiden en ftalaten was geassocieerd met een lager gewicht van de placenta. Hiermee onderbouwen we mogelijk de hypothese dat blootstelling aan chemische stoffen kan leiden tot een suboptimale ontwikkeling van de placenta wat kan resulteren in een verminderde foetale groei. In **Hoofdstuk 2.4** vonden we dat hoge blootstelling aan bisphenol A was geassocieerd met verminderde foetale groei. Vergeleken met vrouwen met lage concentraties bisphenol A in de urine, hadden vrouwen met hoge concentraties een lagere foetale groeisnelheid voor foetaal gewicht en hoofdomtrek. Verder, konden we het effect van de gekozen meetstrategie op de geobserveerde effectmaat onderzoeken. De bisphenol A – foetale groei relatie was afhankelijk van het aantal beschikbare metingen per deelnemer en significante verbanden werden vooral gevonden bij minimaal drie metingen per zwangere vrouw. Met de resultaten van deze studie toonden we aan dat er betere strategieën noodzakelijk zijn om de blootstelling te kwantificeren, en deze resultaten verklaren ook deels waarom sommige studies negatieve bevindingen rapporteren. In **Hoofdstuk 2.5** onderzochten we de hypothese of blootstelling van de ouders aan chemische stoffen het voorkomen van aangeboren hartafwijkingen beïnvloedt. Beroepsmatige blootstelling van de vader aan ftalaten was geassocieerd met een hoger voorkomen van aangeboren hartafwijkingen in het algemeen, en verschillende chemische stoffen, waaronder ftalaten, polychloor stoffen en alkylerende stoffen waren geassocieerd met specifieke fenotypen van aangeboren hartafwijkingen. Verstoring van het epigenetische mechanisme door chemische stoffen kan ten grondslag liggen aan de gevonden effecten van beroepsmatige blootstelling aan chemische stoffen en het optreden van aangeboren hartafwijkingen.

In **Deel 3** van dit proefschrift presenteren we twee studies die de relatie tussen zwaar fysiek werk en zwangerschap onderzoeken. In **Hoofdstuk 3.1** vonden we geen effecten van verschillende beroepsmatige risicofactoren, waaronder zwaar fysiek werk, werkuren per week en blootstelling aan chemische stoffen, op het voorkomen van hypertensieve stoornissen tijdens de zwangerschap. Deze resultaten toonden geen effect van deze beroepsmatige risicofactoren op de maternale cardiovasculaire gezondheid. **Hoofdstuk 3.2** beschrijft dat zwaar fysiek werk, specifiek lange perioden van staan en veel werkuren per week, de groeisnelheden voor foetaal gewicht en hoofdomtrek beïnvloeden. Er werd een negatief effect van lange perioden staan op de groeisnelheid voor foetale hoofdomtrek gevonden. Voor veel werkuren per week werden zowel effecten op foetaal gewicht als hoofdomtrek gevonden. Deze bevindingen geven aan dat zwaar fysiek werk en lange werkuren tijdens de zwangerschap de intrauteriene groei kunnen beïnvloeden. Alhoewel, we vonden geen consistente effecten van deze risicofactoren op ongunstige geboorteuitkomsten.

Deel 4 van dit proefschift richt zich op het gebruik van pijnstillers tijdens verschillende periodes in de zwangerschap en het risico op aangeboren afwijkingen, zoals cryptorchisme en hypospadie in het nageslacht. In **Hoofdstuk 4.1** beschrijven we dat gebruik van pijnstillers tijdens het tweede trimester van de zwangerschap is geassocieerd met een verhoogd risico op cryptorchisme in het mannelijke nageslacht. Omdat een relatief groot aantal zwangere vrouwen paracetamol gebruikt tijdens de zwangerschap, zou de invloed op populatieniveau groot kunnen zijn. We berekenden het populatie attributief risico voor pijnstiller gebruik in het tweede trimester, en indien causaliteit kan worden aangetoond, zou ongeveer 24% van de gevallen van cryptorchisme kunnen worden toegeschreven aan het gebruik van pijnstillers tijdens de zwangerschap. Paracetamol en andere pijnstillers verminderen de productie van androgenen in de ontwikkelende foetus, en hebben zo een nadelige invloed op de androgeen afhankelijke indaling van de testis.

Tenslotte vat **Deel 5** de belangrijkste bevindingen van de studies in dit proefschrift samen en bediscussieerd de methodologische beperkingen en interpretatie van de bevindingen. Suggesties voor toekomstig onderzoek worden besproken.

We kunnen concluderen dat de studies beschreven in dit proefschrift aantonen dat verschillende beroepsmatige en omgevingsgerelateerde risicofactoren verscheidene domeinen van de reproductie kunnen beïnvloeden, zoals de vruchtbaarheid, intrauteriene groei, hypertensieve stoornissen tijdens de zwangerschap, en aangeboren afwijkingen. Verdere studies zijn nodig om deze bevindingen te onderbouwen of te verwerpen en om de onderliggende mechanismen te verklaren. De resultaten van toekomstig onderzoek en de resultaten van de studies uit dit proefschrift kunnen het inzicht vergroten over de negatieve effecten van bepaalde beroepsmatige en omgevingsgerelateerde blootstellingen in het algemeen, en in het bijzonder voor zwangere vrouwen en hun ongeboren kind.

PART 7

LIST OF ABBREVIATIONS
AUTHOR'S AFFILIATIONS
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LIST OF ABBREVIATIONS

AC	Abdominal circumference
AS	Aortic valve stenosis
AVSD	Atrioventricular septal defect
BMI	Body Mass Index
BPA	Bisphenol A
BPD	Biparietal diameter
CHC	Child health care centre
CHD	Congenital heart defect
CI	Confidence interval
CoA	Coarctation of the aorta
COX	Cyclooxygenase
Crea	Creatinine
DCO	Dutch classification of occupations
DDT	Dichloordifenyltrichloorethaan
DEHP	Di(2-ethylhexyl)phthalate
DES	Diethylstilbestrol
DF	Detection frequency
DNA	Desoxyribonucleïnezuur
ED	Endocrine disruptor
EFW	Estimated foetal weight
ER	Estrogen receptor
EU	European Union
FFQ	Food frequency questionnaire
FL	Femur length
FR	Fecundability ratio
GA	Gestational age
GM	Geometric mean
GSD	Geometric standard deviation
HC	Head circumference
HLHS	Hypoplastic left heart syndrome
HR	Hazard ratio
ISSHP	International Society for the Study of Hypertension in Pregnancy
IUGR	Intrauterine growth retardation
JEM	Job-Exposure-Matrix
LBW	Low birth weight
$lnBPA_{CB}$	Log transformed creatinine-based Bisphenol A concentration
LOD	Limit of detection

MCMC	Markov chain monte carlo
MEC	Medical ethics committee
NSAIDS	Non-steroidal anti-inflammatory drugs
OR	Odds ratio
PAF	Population attributable fraction
PAH	Polycyclic aromatic hydrocarbons
PCB	Polychlorinated biphenyl
PE	Preeclampsia
PIH	Pregnancy induced hypertension
PS	Pulmonary valve stenosis
pVSD	Perimembranous ventricular septal defect
RR	Relative risk
SAS	Statistical Analysis System
SBC	Standaard beroepen classificatie
SD	Standard deviation
SDS	Standard deviation score
SES	Socio economic status
SGA	Small-for-gestational-age
SOC	Standard occupational classification
SPSS	Statistical Package Social Sciences
TCPy	3,5,6-trichloro-2-pyridinol
TDS	Testicular dysgenesis syndrome
TGA	Transposition of the great arteries
TOF	Tetralogy of Fallot
TTP	Time to pregnancy
WAZ	Weight-for-age z-score
WHO	World health organisation

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Matthew P Longnecker

The Department of Urban Environment and Safety, TNO (Netherlands Organisation for Applied Scientific Research), Utrecht, The Netherlands
Frank H Pierik

PUBLICATION LIST

1. Occupational exposure to chemical substances and time to pregnancy: a systematic review.
   Snijder CA, te Velde E, Roeleveld N, Burdorf A.
   Human Reproduction Update 2012;18:284-300.
   (IF 8.76)

2. Occupational exposure to endocrine disruptors and time to pregnancy among couples in a large birth cohort study: the Generation R Study.
   Snijder CA, Brouwers MM, Jaddoe VW, Hofman A, Roeleveld N, Burdorf A.
   Fertility and Sterility 2011;95:2067-2072.
   (IF 3.96)

3. Occupational exposure to chemicals and fetal growth: the Generation R Study.
   Snijder CA, Roeleveld N, te Velde E, Steegers EA, Raat H, Hofman A, Jaddoe VW, Burdorf A.
   Human Reproduction 2012;27:910-920.
   (IF 4.36)

4. Prenatal exposure to Bisphenol A and fetal growth: the Generation R Study.
   Snijder CA, Heederik D, Pierik FH, Hofman A, Jaddoe VW, Koch HM, Longnecker MP, Burdorf A.
   Environmental Health Perspectives, provisionally accepted for publication.
   (IF 7.03)

5. Congenital heart defects and parental occupational exposure to chemicals.
   Snijder CA, Vlot IJ, Burdorf A, Obermann-Borst SA, Helbing WA, Wildhagen MF, Steegers EA, Steegers-Theunissen RP.
   Human Reproduction 2012;27:1510-1517.
   (IF 4.36)

6. Work-related maternal risk factors and the risk of pregnancy induced hypertension and preeclampsia during pregnancy. The Generation R Study.
   Nugteren JJ, **Snijder CA**, Hofman A, Jaddoe VW, Steegers EA, Burdorf A.
   PLoS One 2012;7:e39263.
   (IF 4.41)

7. Physically demanding work, fetal growth, and the risk of adverse birth outcomes. The Generation R Study.
   Snijder CA, Brand T, Jaddoe V, Hofman A, Mackenbach JP, Steegers EA, Burdorf A.
   Occupational and Environmental Medicine 2012;69:543-550.
   (IF 3.49)

8. Intrauterine exposure to mild analgesics during pregnancy and the occurrence of cryptorchidism and hypospadia in the offspring: the Generation R Study.
   Snijder CA, Kortenkamp A, Steegers EA, Jaddoe VW, Hofman A, Hass U, Burdorf A
   Human Reproduction 2012;27:1191-1201.
   (IF 4.36)

Other publications

9. Levels of circulating endothelial cells in normotensive and severe preeclamptic pregnancies.
   Strijbos MH, **Snijder CA**, Kraan J, Lamers CH, Gratama JW, Duvekot JJ.
   Cytometry B Clinical Cytometry 2010;78:382-386.
   (IF 1.96)

10. Thromboprophylaxis and bleeding complications after cesarean section.
    Snijder CA, Cornette JM, Hop WC, Kruip MJ, Duvekot JJ.
    Acta Obstetricia Gynecologica Scandinavica 2012;91:560-565.
    (IF 1.86)

11. Reliability of urinary Bisphenol A concentrations measured during pregnancy in the Generation R Study.
    Jusko TA, Shaw PA, **Snijder CA**, Pierik FH, Koch H, Hauser R, Jaddoe VWV, Burdorf A, Hofman A, Tiemeier H, Longnecker MP.
    Submitted to *Environmental Research*

ABOUT THE AUTHOR

Claudia Snijder werd geboren op 4 mei 1983, te Rotterdam. Zij is de dochter van Cornelis Snijder en Tineke Snijder-Munter. Na het behalen van het VWO diploma aan het Farel College te Ridderkerk, begon zij in 2002 met de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. Tijdens haar studie deed zij haar wetenschappelijke stage op de afdeling obstetrie en gynaecologie onder leiding van Dr. J.J. Duvekot naar nabloedingen bij patiënten die een keizersnede hadden ondergaan en endotheel cellen bij patiënten met preeclampsie. In december 2008 behaalde zij haar artsexamen (cum laude).

Na twee maanden als ANIOS gynaecologie/verloskunde in het Ikazia ziekenhuis te hebben gewerkt startte zij in maart 2009 met haar promotieonderzoek naar de effecten van werk en omgeving op de voortplanting. Dit proefschrift toont aan dat blootstelling aan bepaalde chemische stoffen op het werk, alsmede zwaar fysiek werk, van invloed kan zijn op verschillende aspecten van de voortplanting. Dit onderzoek was een samenwerking tussen de afdelingen Maatschappelijke Gezondheidszorg en Generation R.

Op 16 april startte zij als ANIOS gynaecologie/verloskunde in het Reinier de Graaf Gasthuis te Delft, opleider Dr. H. Bremer. In juni 2012 werd zij aangenomen voor de opleiding tot Gynaecoloog en op 1 augustus startte zij als AIOS in het Bronovo ziekenhuis te Den Haag, opleider Dr. C.A.G. Holleboom.

PHD PORTFOLIO

Name PhD student:	C.A.Snijder
Erasmus MC department:	Public Health
Research school:	NIHES
PhD Period:	March 2009 – April 2012
Promotor:	A. Burdorf
Supervisor:	A. Burdorf

1. PhD Training	**Year**	**Workload (ECTS)**
General academic skills		
Methodologie van patient gebonden onderzoek en voorbereiding subsidie aanvragen, Erasmus MC Rotterdam	2008	5.0
Research skills		
Master of Science in Epidemiology, Netherlands Institute for Health Sciences (Nihes), Rotterdam, The Netherlands	2009-2012	35.0
(Inter) National Conferences – participation and presentations		
Najaarsvergadering sectie Teratologie en Reproductietoxicologie, TNO Zeist. Oral presentation: HAVEN Study: Periconceptional parental occupational exposure to endocrine disruptors and the risk of congenital heart defects in offspring. The HAVEN study.	2009	0.8
SGI 2010, Society for Gynecologic Investigation, 57th Annual Scientific Meeting 'Epigenetic Regulation' in Orlando, Florida, USA. Poster presentation: Periconceptional exposure to Endocrine Disruptors and the association with Congenital Heart Defects. The HAVEN Study.	2010	1.0
EPICOH – Medichem 2010, Occupational Health under Globalisation and New Technology, 21th Annual Scientific Meeting in Taipei, Taiwan Oral presentations:	2010	2.0

1. Periconceptional parental Occupational exposure to Endocrine Disruptors and Congenital Heart Defects – The HAVEN Study –
2. Occupational exposure to Endocrine Disruptors of the Parents-to-be and Time-to-pregnancy. The Generation R Study.

Enrieco meeting, Environmental health risks in European birth cohorts, 2nd ENRIECO meeting, Utrecht, May 2010.	2010	0.5
Gynaecongres Breda, 37e Gynaecongres, Nederlandse Vereniging voor Obstetrie en Gynaecologie. Chassétheater Breda, Juni 2010. Oral presentation: Beroepsmatige blootstelling van ouders in depericonceptionele periode aan endocrien verstorende stoffen encongenitale hartafwijkingen – De Haven Studie –	2010	1.0
COW 2011, 6th Copenhagen Workshop on Endocrine Disruptors, Copenhagen, Denemarken. April, 2011.	2011	1.0
Najaarsvergadering werkgroep Reproductie en Arbeid, Coronel Institute for Occupational Health, Academin Medical Centre Amsterdam, The Netherlands. Oral presentation: Occupational exposures and time to pregnancy.	2011	0.8
Research meeting, department of Public Health, Erasmus MC, Rotterdam. Oral presentation: Work, environment and reproduction.	2012	1.0

Seminars and workshops

Attending seminars of the Department of Public health	2009-2012	1.0
Workshop urinary biomarkers, London, Centre for Toxicology	2010	1.0
Attending the Generation R Study Group research meetings	2009-2011	1.0
Attending RCOG onderzoeksdag / Wladimiroff Symposium, Erasmus MC.	2009-2012	0.8
Wetenschapsdag gynaecologie en urologie, Erasmus MC.	2009	0.8
Symposium "New imaging and developmental concepts in early pregnancy" Erasmus MC.	2009	0.8

2. Teaching Activities

Supervising Master's Thesis

Ingrid Vlot, Medical student, Erasmus University, Rotterdam Project title: Parental occupation and congenital heart defects.	2009	2.0
Jaap Jan Nugteren, Medical student, Erasmus University, Rotterdam Project title: Occupational risk factors and hypertensive disorders during pregnancy.	2011	2.0

Other skills

Review for Environmental Health	2011
Review for Human Reproduction	2011
Review for Human Reproduction	2012
Review for Human Reproduction	2012

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