Maternal occupational exposure and congenital anomalies

Spinder, Nynke

DOI:
10.33612/diss.136730422

Maternal occupational exposure and congenital anomalies

Nynke Spinder

Cover design by Nynke Spinder
Layout and design: Eduard Boxem | www.persoonlijkproefschrift.nl
Printing: Ridderprint | www.ridderprint.nl

rijksuniversiteit
groningen

Maternal occupational exposure and congenital anomalies

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 18 november 2020 om 14.30 uur

door

Nynke Spinder

geboren op 21 februari 1993
te Amsterdam

Promotores
Prof. dr. H.M. Boezen
Prof. dr. H. Kromhout

Copromotores
Dr. H.E.K. de Walle
Dr. J.E.H. van Kammen-Bergman

Beoordelingscommissie
Prof. dr. J.P. Bonde
Prof. dr. A. Hoek
Prof. dr. I.M. van Langen

Paranimfen
Tine Hoogterp
Tjerk Hidde Hylkema

CONTENTS

CHAPTER 1

General introduction

In the early sixties the world was shocked by the thalidomide (Softenon) "epidemic". This drug, used as a sedative and in treatment of nausea in pregnant women, resulted in births of infants affected by severe congenital anomalies such as limb reduction defects [1-3]. In the late sixties, Vietnamese citizens and Vietnam War veterans reported increased rates of congenital anomalies in their offspring after exposure to Agent Orange, an herbicide used to destroy dense jungle get advance of the Vietnamese guerrillas during the war [4]. In 2015, the Zika virus spread rapidly throughout South America and led to an increased number of infants born with microcephaly in Brazil [5]. Recently, possible new congenital anomaly "epidemics" were reported in the media, such as an increased prevalence of congenital anomalies in Limburg, the Netherlands [6], three infants born with deformed hands in Germany [7], and increased rates of limb reduction defects in France [8]. Despite wide-spread rumours regarding the causes of those increases in the numbers of infants born with congenital anomalies, no scientific evidence for a true increase and no specific cause have been identified as of yet [9]. These events emphasize the need for congenital anomaly registries and systematic epidemiological research. Through monitoring of the prevalence of congenital anomalies, the true increases in congenital anomalies and potential risk factors for rare and specific congenital anomalies can be studied.

CONGENITAL ANOMALIES

Worldwide, congenital anomalies are one of the main causes of neonatal and infant mortality [10-12]. One in 33 infants is affected by a congenital anomaly, resulting in more than 100,000 affected births in Europe each year. The majority of these births result in live-born infants, about 2% are stillbirths, and around 20% of the pregnancies are terminated because of a congenital anomaly (EUROCAT 2011-2017)[13]. If new-borns survive, they often have to deal with long-term disabilities or need surgery. Depending on the type of anomaly, this could be one relatively simple surgical intervention or several complex interventions. This not only impacts the life of the child, but also their families, healthcare systems and society. In this thesis, congenital anomalies are defined as anomalies that develop during intrauterine life. In Figure 1 the distribution of the different types of congenital anomalies are displayed. The most common congenital anomalies are congenital heart defects (30%), followed by chromosomal disorders (17%), limb anomalies (17%) and urinary anomalies (14%).

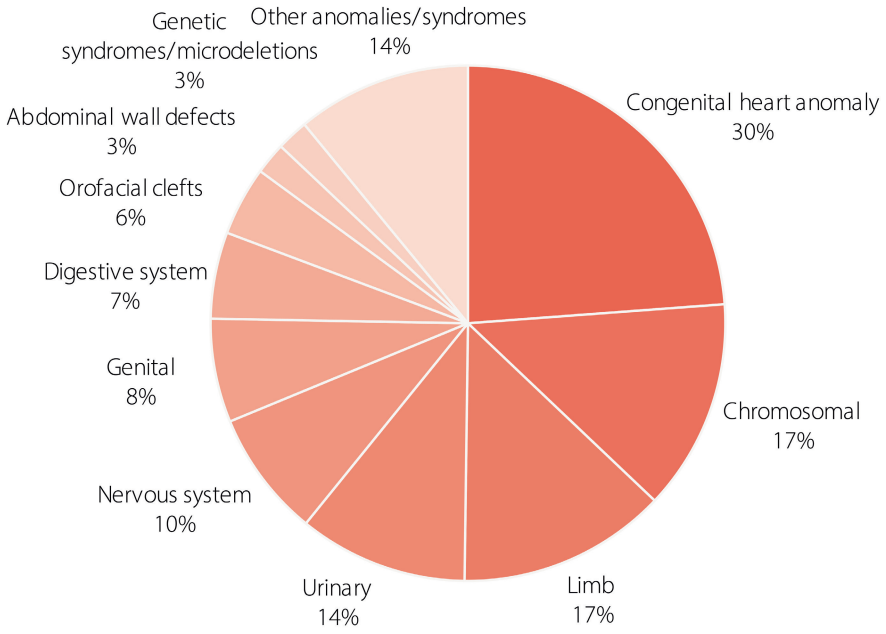


Figure 1 | Distribution of anomaly groups: Eurocat – Prevalence 2011-2017 [13]

RISK FACTORS

Congenital anomalies can be caused by genetic factors, environmental factors, or a combination of both. Primary foetal development and organogenesis starts directly after conception (Box 1). Since the foetus is vulnerable during this process, external exposures during the periconceptional period (one month before conception through three months after conception) can affect embryological development. Maternal medical conditions that can increase the risk of congenital anomalies are being overweight, having poorly regulated maternal diabetes, phenylketonuria, and infections during the periconceptional period (e.g. cytomegalovirus, rubella, and Zika virus) [14-16]. In addition, the use of specific drugs such as anticonvulsants, antidepressants, cholesterol-lowering agents, angiotensin-converting enzyme inhibitors, and folic acid antagonists may result in an increased risk of congenital anomalies [15,17]. On the other hand, pregnant women and women who want to become pregnant are advised to take folic acid supplementation starting four weeks prior to conception through 10 weeks after conception because folic acid supplementation

reduces the risk of neural tube defects and congenital heart defects [18,19]. Several lifestyle factors can also increase the risk of congenital anomalies in offspring, including maternal and paternal smoking, alcohol consumption, and illicit drug use [15,20,21]. In the general environment women can be exposed to air pollutants that can increase the risk of congenital anomalies [22]. Exposures to teratogens can also occur in the workplace and could be an important risk factor for congenital anomalies.

Box 1 - The critical time after conception (Embryology)

After the oocyte is fertilized by the spermatozoa, the zygote is formed. The zygote undergoes cleavage, which results in the blastula (day 5 after conception). This blastula (1.5-2.0 mm) implants in the endometrium of the uterus and undergoes gastrulation (day 7). This process results in the formation of three germ layers: mesoderm, ectoderm, and endoderm. Each of these germ layers differentiates into different organ systems. Three weeks after conception, body folding starts, and the embryo will have a shape that is starting to resemble that of the adult. At the same time, organogenesis takes place and limbs develop. The ectoderm forms the nervous system and skin cells, the mesoderm gives rise to the muscle cells and connective tissue in the body, and the endoderm forms the digestive system and other internal organs. The heart begins to beat at week 4, and the primary organs are formed 8 weeks after conception [67].

OCCUPATIONAL EXPOSURE

As an increasing number of women are working during their reproductive years, knowledge about occupational risk factors is important for employers and employees, as well as freelancers and independent contractors. Nowadays, more than 80% of Dutch women are participating in the labour force, compared to just 50% 40 years ago [23]. Although Dutch women work fewer hours than women anywhere else in Europe, the number of hours women are working in the Netherlands is increasing over time [24,25].

During their work, women can be exposed to a wide range of factors that might affect their reproductive health and pregnancy outcomes. Exposure to various chemicals, such as solvents, pesticides and metals, have been associated with reduced fertility, prolonged

time to pregnancy, increased risks of spontaneous abortions, prematurity, and reduced birth weight [26-29]. A variety of occupational exposures are discussed in this thesis: mineral dust, biological dust, gases and fumes, and more specific solvents, pesticides, metals, and other endocrine disrupting chemicals (EDCs) like polycyclic aromatic hydrocarbons, phthalates, benzophenones, parabens, and siloxanes. Routes of exposure are inhalation, dermal absorption, and ingestion. Occupational exposure to mineral and biological dusts, and gases and fumes occurs in manufacturing, construction, cleaning, food processing, and agriculture. Women can be exposed to solvents when working in healthcare, in beauty or hairdressing salons, or in cleaning occupations. Pesticides are commonly used among farmers and other agricultural workers. Most women occupationally exposed to metals are working in assembly of electronic equipment or mechanical machinery.

As embryo development and organogenesis takes place in utero in the first trimester, it is important to assess maternal occupational exposure during the periconceptional period. Men can be exposed to the same agents as women during their work, and previous studies suggest that paternal occupational exposure to chemicals can increase the risk of congenital anomalies such as neural tube defects, congenital heart defects, and hypospadias [30-33]. However, paternal occupational exposure can affect spermatogenesis before conception, while maternal occupational exposure can affect the oocyte before conception as well as the embryo directly during foetal development. This thesis therefore focuses only on maternal occupational exposure.

AIMS

Since more and more women are participating in the labour force and the etiological processes of many congenital anomalies are not yet understood, it is important to identify occupational risk factors in order to protect women who want to become pregnant or are pregnant and their offspring. Particularly because being pregnant with or having an infant with a congenital anomaly has a large impact on infant mortality and morbidity, families, and society. Therefore, the aim of this thesis is to examine the association between maternal occupational exposures during the periconceptional period and congenital anomalies in the offspring. First, an overview of the literature will be presented (Chapter 2). In the next chapters, maternal occupational exposure in relation to specific congenital anomalies is studied (Chapters 3-6). The specific congenital anomalies examined, study

populations, and exposure assessment methods used in this thesis are described in the following paragraphs.

Congenital anomalies studied in this thesis

Five subgroups of congenital anomalies are examined in this thesis (Figure 2). These are common congenital anomalies, and it is possible that occupational exposure can influence their development because these anomalies are of heterogenic origin.

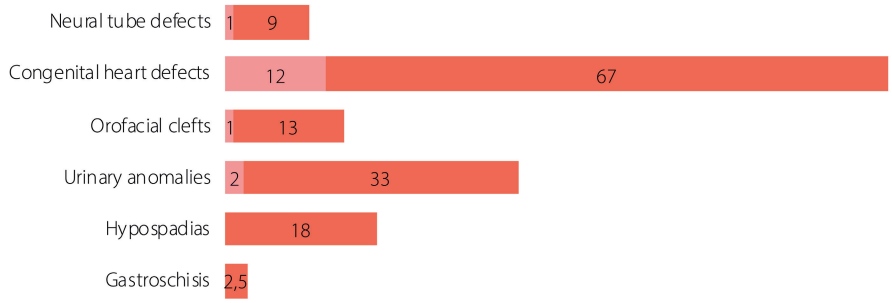


Figure 2 | Prevalence of congenital anomalies (genetic origin and non-genetic origin) in Europe in 2011-2017 (per 10,000 births) [13]

Neural tube defects

Neural tube defects are defects of the central nervous system that develop when the neural tube fails to close during the third and fourth week after conception. Examples of neural tube defects are anencephaly, which is not compatible with life, and spina bifida, which introduces several developmental and neurological problems depending on the site and type of the defect. In Europe, 10 per 10,000 births are affected by a neural tube defect (Figure 2). Many factors are involved in the abnormal closure of the neural tube (e.g. folic acid usage) [34]. Several studies have assessed the association between maternal occupational exposures and neural tube defects in offspring, but no clear conclusion could be drawn regarding the effect of occupational exposures on development of neural tube defects.

Congenital heart defects

Congenital heart defects are the most common congenital anomalies. In Europe, 79 infants and foetuses per 10,000 births are affected (Figure 2). Subgroups of congenital heart defects are anatomically, clinically, epidemiologically, and developmentally

heterogeneous. Examples of congenital heart defect subgroups are conotruncal defects, left or right ventricular outflow tract defects, and septal defects. It is important to assess the subgroups of congenital heart defects because these defects might differ in aetiology. Previous studies have suggested associations between maternal occupational exposure to solvents or pesticides and specific congenital heart defects [35,36]. However, for most studies it has not been possible to assess the association between maternal occupational exposure and subgroups of congenital heart defects because most studies have only included a small number of cases.

Orofacial clefts

Orofacial clefts are malformations that result from failure of fusion of the lip and/or palate. The European prevalence of orofacial clefts is 14 per 10,000 births (Figure 2). The aetiology of orofacial clefts is not fully understood. It is known that the aetiology of the two subtypes of orofacial clefts, cleft lip with or without cleft palate and cleft palate, are different. Several studies have suggested that maternal occupational exposure to solvents or pesticides can increase the risk of orofacial clefts in offspring [37-43], and one study suggested an association between exposure to metals and orofacial clefts [44].

Urogenital defects

Urogenital anomalies are congenital anomalies representing any defect in the organs and tissues responsible for the formation and excretion of urine. Anomalies can be malformations of the renal parenchyma, anomalies of the urinary collecting system, abnormal embryonic migration of kidneys, or hypospadias. In hypospadias, the most common genital anomaly, the urethral opening is located at the ventral side of the penis. The prevalence of kidney and urinary collecting system anomalies in Europe is 35 per 10,000 births, whereas 18 per 10,000 births are affected by hypospadias (Figure 2). It has been hypothesised that exposure to EDCs could influence the hormonal activity and adversely affect foetal development of the urogenital tract [45]. Several studies have reported associations between maternal occupational exposure to EDCs and hypospadias [46-49]. Studies regarding occupational exposure in relation to urinary anomalies are scarce.

Gastroschisis

Gastroschisis is a severe anomaly of the abdominal wall that involves a full-thickness para-umbilical defect through which intestines and other organs may herniate without a covering membrane. Approximately 4.5 per 10,000 births are affected by a gastroschisis

in the United States [50], compared to 2.5 per 10,000 births in Europe (Figure 2). It has been hypothesized that gastroschisis develops due to rupture or non-closure of the membrane covering the umbilical ring between 8 and 11 weeks after fertilization [51,52]. Only one study has reported an association between maternal occupational exposure to solvents and gastroschisis [53].

Study populations

In this thesis, the association between maternal occupational exposure and congenital anomalies in offspring has been explored by conducting several case–control studies. Three different data sources have been used for these studies: Eurocat NNL and Lifelines from the Netherlands and the National Birth Defects Prevention Study (NBDPS) from the United States.

Eurocat NNL

The European Concerted Action on Congenital Anomalies and Twins Northern Netherlands (Eurocat NNL) is a population-based registry of congenital anomalies [54]. This registry was founded in 1981 as part of a European network of congenital anomaly registries. The Eurocat NNL registry currently monitors about 16,000 births annually in the northern Dutch provinces of Groningen, Friesland and Drenthe. In addition to live births (up to 10 years of age at notification), Eurocat NNL also registers stillbirths, miscarriages, and pregnancies terminated because of congenital anomalies. These cases are reported by midwives, child healthcare physicians, and medical specialists. Additionally, sources are actively sought by registry workers to find children or pregnancies eligible for registration. The Eurocat NNL database contains detailed and high-quality information on approximately 18,000 children or foetuses with congenital anomalies. Detailed medical information is available for each case, and all cases were coded by trained registry staff according to international coding guidelines [55]. Since 1997, parents have also been asked to complete a questionnaire. Information is collected regarding the pregnancy, obstetric and medical history, demographic characteristics, use of medication, and occupation and lifestyle during the periconceptional period. Data from Eurocat NNL is used in Chapters 3-5.

Lifelines

Lifelines is a three-generation prospective cohort study that is following 167,000 participants over a 30-year period in the same Northern Dutch region as Eurocat NNL [56]. The aim of Lifelines is to obtain insight into healthy ageing. Participants were invited

through their general practitioners. Lifelines participants (between 18 and 65 years old) were asked to invite their offspring and parents to create a three-generation cohort. Their children could participate if they were between 6 months and 18 years old. We selected infants without a congenital anomaly from the Lifelines cohort as controls for the studies in Chapters 4 and 5. Parents of participating children completed a questionnaire regarding the pregnancy, their health, occupation, and lifestyle during pregnancy, childbirth, and health of their child in the first six months of life.

National Birth Defects Prevention Study

The National Birth Defects Prevention Study (NBDPS) is a large population-based multicentre case–control study of major structural congenital anomalies in the United States [57]. Pregnancies with estimated delivery dates between 1997 and 2011 from ten states were included. All states included live-born cases, and most states also included cases of stillbirths and terminated pregnancies with a prenatal diagnosis of congenital anomalies. Cases were ascertained by the participating states' congenital anomalies surveillance systems up to two years after delivery. Clinical information extracted from medical records was reviewed by a clinical geneticist at each centre using a systematic study-wide classification protocol to confirm eligibility. Controls were live-born infants without major congenital anomalies selected randomly from either vital records or hospital birth records from the same geographical region and time period as cases. Women who participated in the NBDPS completed a computer-assisted telephone interview. During this interview, mothers were asked to report information about demographics, medication use, occupational history, and their lifestyle during pregnancy and the three months preceding pregnancy. Data from the NBDPS is used in Chapters 6.

Occupational exposure assessment

Different methods have been developed to assess occupational exposures in epidemiological studies [58]. In this thesis, two occupational exposure assessment methods have been used: job-exposure matrices (JEMs) and individual expert-based assessments by occupational hygienists. When using a JEM, descriptions of jobs held early in pregnancy are coded using the International Standard Classification of Occupations 1988 (ISCO88) [59]. These job codes are then translated into occupational exposure using a JEM designed by occupational hygienists. In Chapters 3 and 5, the ALOHA+ JEM was used to assess exposure to organic and mineral dusts, solvents, pesticides, metals, and gases and fumes [60,61]. In Chapter 4, a JEM assessing occupational exposure to chemicals known for their endocrine-

disrupting effect was used [62,63]. In Chapter 6, individual expert-based assessments by occupational hygienists were performed. Occupational experts can perform occupational exposure assessment for a variety of occupational exposures based on occupational histories that include job title, employer name, what the company makes or does, primary tasks and duties, a description of chemicals and machines handled on the job, dates of employment, and hours and days worked per week [64].

Both methods involve occupational hygienists, who by training have an understanding of exposure sources and pathways leading to occupational exposure, and both methods reduce the risk of recall bias and misclassification compared to the traditional method of using self-reported exposure in case–control studies [29,65]. The JEM approach is less time-consuming and cheaper, but assigns exposure at job level and by definition will not be able to make a distinction between the exposures of two women reporting the same job. This would be feasible with an individual-based expert assessment, but might result in differential misclassification given that it is based on self-reporting of tasks and duties and chemicals used. Comparisons of both methods in a multi-centre study demonstrated little advantage of individual exposure assessment when compared to a JEM [66].

Measuring occupational exposures directly is almost never feasible because measurements would have to be performed prospectively during the periconceptional period. This is almost impossible since most congenital anomalies are discovered after the periconceptional period, and prevalence of congenital anomalies is low. Biomonitoring of chemicals with a long half-life in cord blood would be a possibility, but again a large number of infants with congenital anomalies has to be included.

OUTLINE OF THIS THESIS

Chapter 2 provides an overview of the literature on the effects of maternal occupational exposure to solvents, pesticides and metals on several anomalies in offspring including neural tube defects, congenital heart defects, orofacial clefts, and hypospadias.

In Chapter 3, the association between maternal periconceptional occupational exposure and orofacial clefts in offspring is examined using a JEM and data from the Eurocat NNL registry.

In Chapter 4, the association between maternal occupational exposure early in pregnancy and urogenital anomalies in offspring is assessed using a JEM and data from Eurocat NNL and Lifelines.

In Chapter 5, the effect of maternal occupational exposure early in pregnancy on congenital heart defects in offspring is studied using a JEM and data from Eurocat NNL and Lifelines.

In Chapter 6, the effect of maternal occupational exposure to solvents on gastroschisis in offspring is examined using industrial hygienists and data from the NBDPS, United States.

In Chapter 7, the results of this thesis are summarised and discussed, and implications for further research and preventive policies are presented.

REFERENCES

1. Lenz W, Pfeiffer RA, Kosenow W, Hayman DJ. Thalidomide and congenital abnormalities. Lancet. 1962;279(7219):45-46.
2. McBride WG. Thalidomide and congenital abnormalities. Lancet. 1961;2(1358):90927-90928.
3. Vargesson N. Thalidomide-induced teratogenesis: History and mechanisms. Birth Defects Res C Embryo Today. 2015;105(2):140-156.
4. Ngo AD, Taylor R, Roberts CL, Nguyen TV. Association between agent orange and birth defects: Systematic review and meta-analysis. Int J Epidemiol. 2006;35(5):1220-1230.
5. Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects--reviewing the evidence for causality. N Engl J Med. 2016;374(20):1981-1987.
6. Nederlandse Omroep Stichting. Meer aangeboren afwijkingen in Limburg. 05/25, 2018. Available from: https://nos.nl/artikel/2233478-meer-aangeboren-afwijkingen-in-limburg.html.
7. Sankt Marien-Hospital Buer. Geburten von babys mit handdeformitäten: Stellungnahme zu aktuellen beiträgen in diversen medien. 2019. Available from: http://marienhospital-buer.de/detailseite/?tx_ttnews%5Btt_news%5D=1640&cHash=bf72b8630fba38053b6cccbe3164283d.
8. Daily Mail. Scientists are baffled by spatter of babies born without hands or arms in france, as investigation fails to discover a cause. 7/12, 2019. Available from: https://www.dailymail.co.uk/news/article-7242059/Scientists-baffled-babies-born-without-hands-arms-France-probe-fails-discover-cause.html.
9. Benachi A, Babajko C, Barjat-Raia T, et al. Initial report on transverse agenesis of the upper limbs (TAUL). Sante Publique France. 2019.
10. World Health Organization. Causes of child mortality, 2017. https://www.who.int/gho/child_health/mortality/causes/en/. Updated 2017. Accessed 1/15/, 2020.
11. Murphy SL, Xu J, Kochanek KD, Arias E. Mortality in the united states, 2017. NCHS data brief. 2018(328):1-8.
12. Boyle B, Addor MC, Arriola L, et al. Estimating global burden of disease due to congenital anomaly: An analysis of european data. Arch Dis Child Fetal Neonatal Ed. 2018;103(1):F22-F28.
13. EUROCAT. Prevalence charts and tables. https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-data/prevalence. Updated 2017. Accessed 1/15, 2020.
14. Stothard KJ, Tennant PW, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: A systematic review and meta-analysis. JAMA. 2009;301(6):636-650.
15. Harris BS, Bishop KC, Kemeny HR, Walker JS, Rhee E, Kuller JA. Risk factors for birth defects. Obstet Gynecol Surv. 2017;72(2):123-135.
16. Johnson KE. Overview of TORCH infections. https://www.uptodate.com/contents/overview-of-torch-infections?search=torch&source=search_result&selectedTitle=1~29&usage_type=default&display_rank=1#H28. Updated 2019. Accessed 1/21, 2020.
17. Gao SY, Wu QJ, Sun C, et al. Selective serotonin reuptake inhibitor use during early pregnancy and congenital malformations: A systematic review and meta-analysis of cohort studies of more than 9 million births. BMC Med. 2018;16(1):205-5.

18. Feng Y, Wang S, Chen R, Tong X, Wu Z, Mo X. Maternal folic acid supplementation and the risk of congenital heart defects in offspring: A meta-analysis of epidemiological observational studies. Sci Rep. 2015;5:8506.

19. Khoshnood B, Loane M, de Walle H, et al. Long term trends in prevalence of neural tube defects in europe: Population based study. BMJ. 2015;351:h5949.

20. Nicoletti D, Appel LD, Siedersberger Neto P, Guimaraes GW, Zhang L. Maternal smoking during pregnancy and birth defects in children: A systematic review with meta-analysis. Cad Saude Publica. 2014;30(12):2491-2529.

21. Zhao L, Chen L, Yang T, et al. Parental smoking and the risk of congenital heart defects in offspring: An updated meta-analysis of observational studies. Eur J Prev Cardiol. 2019:2047487319831367.

22. Vrijheid M, Martinez D, Manzanares S, et al. Ambient air pollution and risk of congenital anomalies: A systematic review and meta-analysis. Environ Health Perspect. 2011;119(5):598-606.

23. Centraal Bureau voor de Statistiek, (CBS). Arbeidsdeelname, vanaf 1969. https://opendata.cbs.nl/statline/#/CBS/nl/dataset/83752NED/table?fromstatweb. Updated 2019. Accessed 1/17, 2020.

24. Thor (CBS) Jv, Portegijs (SCP) W, Hermans (CBS) B. Gaan vrouwen steeds meer werken? Emancipatiemonitor: 2018. 2018. https://digitaal.scp.nl/emancipatiemonitor2018/gaan-vrouwen-steeds-meer-werken.

25. Sociaal en Cultureel Planbureau. Werken aan de start: Jonge vrouwen en mannen op de arbeidsmarkt. . 2018:83-84.

26. Kumar S. Occupational exposure associated with reproductive dysfunction. J Occup Health. 2004;46(1):1-19.

27. Burdorf A, Figà-Talamanca I, Jensen TK, Thulstrup AM. Effects of occupational exposure on the reproductive system: Core evidence and practical implications. Occup Med (Lond). 2006;56(8):516-520.

28. Figa-Talamanca I. Occupational risk factors and reproductive health of women. Occup Med (Lond). 2006;56(8):521-531.

29. Snijder CA, te Velde E, Roeleveld N, Burdorf A. Occupational exposure to chemical substances and time to pregnancy: A systematic review. Hum Reprod Update. 2012;18(3):284-300.

30. Pettigrew SM, Bell EM, Van Zutphen AR, et al. Paternal and joint parental occupational pesticide exposure and spina bifida in the national birth defects prevention study, 1997 to 2002. Birth Defects Res A Clin Mol Teratol. 2016;106(11):963-971.

31. Kalfa N, Paris F, Philibert P, et al. Is hypospadias associated with prenatal exposure to endocrine disruptors? A french collaborative controlled study of a cohort of 300 consecutive children without genetic defect. Eur Urol. 2015;68(6):1023-1030.

32. Wang C, Zhan Y, Wang F, et al. Parental occupational exposures to endocrine disruptors and the risk of simple isolated congenital heart defects. Pediatr Cardiol. 2015;36(5):1024-1037.

33. Suhl J, Romitti PA, Rocheleau C, et al. Parental occupational pesticide exposure and nonsyndromic orofacial clefts. J Occup Environ Hyg. 2018;15(9):641-653.

34. Avagliano L, Massa V, George TM, Qureshy S, Bulfamante GP, Finnell RH. Overview on neural tube defects: From development to physical characteristics. Birth defects research. 2019;111(19):1455-1467.

35. Gilboa SM, Desrosiers TA, Lawson C, et al. Association between maternal occupational exposure to organic solvents and congenital heart defects, national birth defects prevention study, 1997-2002. Occup Environ Med. 2012;69(9):628-635.

36. Rocheleau CM, Bertke SJ, Lawson CC, et al. Maternal occupational pesticide exposure and risk of congenital heart defects in the national birth defects prevention study. Birth Defects Res Part A Clin Mol Teratol. 2015;103(10):823-833.

37. Desrosiers TA, Lawson CC, Meyer RE, et al. Maternal occupational exposure to organic solvents during early pregnancy and risks of neural tube defects and orofacial clefts. Occup Environ Med. 2012;69(7):493-499.

38. Garlantezec R, Monfort C, Rouget F, Cordier S. Maternal occupational exposure to solvents and congenital malformations: A prospective study in the general population. Occup Environ Med. 2009;66(7):456-463.

39. Lorente C, Cordier S, Bergeret A, et al. Maternal occupational risk factors for oral clefts. Scand J Work Environ Health. 2000;26(2):137-145.

40. Laumon B, Martin JL, Bertucat I, Verney MP, Robert E. Exposure to organic solvents during pregnancy and oral clefts: A case-control study. Reprod Toxicol. 1996;10(1):15-19.

41. Romitti PA, Herring AM, Dennis LK, Wong-Gibbons D. Meta-analysis: Pesticides and orofacial clefts. Cleft Palate-Craniofac J. 2007;44(4):358-365.

42. Holmberg PC, Hernberg S, Kurppa K. Oral clefts and organic solvent exposure during pregnancy. Int Arch Occup Environ Health. 1982;50(4):371-376.

43. Chevrier C, Dananché B, Bahuau M, et al. Occupational exposure to organic solvent mixtures during pregnancy and the risk of non-syndromic oral clefts. Occup Environ Med. 2006;63(9):617-623.

44. Hao Y, Tian S, Jiao X, et al. Association of parental environmental exposures and supplementation intake with risk of nonsyndromic orofacial clefts: A case-control study in heilongjiang province, china. Nutrients. 2015;7(9):7172-7184.

45. Yiee JH, Baskin LS. Environmental factors in genitourinary development. J Urol. 2010;184(1):34-41.

46. Haraux E, Braun K, Buisson P, et al. Maternal exposure to domestic hair cosmetics and occupational endocrine disruptors is associated with a higher risk of hypospadias in the offspring. Int J Environ Res Public Health. 2017;14(1).

47. Kalfa N, Philibert P, Broussous S, et al. Isolated hypospadias and exposure to endocrine disrupting chemicals during pregnancy: A multi-institutional controlled study in a high prevalence area. Horm Res Paediatr. 2014;82:107.

48. Rocheleau CM, Romitti PA, Dennis LK. Pesticides and hypospadias: A meta-analysis. J Pediatr Urol. 2009;5(1):17-24.

49. Warembourg C, Botton J, Lelong N, et al. Prenatal exposure to glycol ethers and cryptorchidism and hypospadias: A nested case-control study. Occup Environ Med. 2018;75(1):59-65.

50. Short TD, Stallings EB, Isenburg J, et al. Gastroschisis trends and ecologic link to opioid prescription rates - united states, 2006-2015. MMWR Morb Mortal Wkly Rep. 2019;68(2):31-36.

51. Bargy F, Beaudoin S. Comprehensive developmental mechanisms in gastroschisis. Fetal Diagn Ther. 2014;36(3):223-230.

52. Opitz JM, Feldkamp ML, Botto LD. An evolutionary and developmental biology approach to gastroschisis. Birth Defects Res. 2019;111(6):294-311.

53. Torfs CP, Katz EA, Bateson TF, Lam PK, Curry CJ. Maternal medications and environmental exposures as risk factors for gastroschisis. Teratology. 1996;54(2):84-92.

54. Eurocat Northern Netherlands. Registry description. https://www.umcg.nl/SiteCollectionDocuments/Zorg/Ouders/Eurocat/Registry_description_NNL_final_version_jan2018.pdf. Updated 2018. Accessed 1/21, 2020.

55. European Surveillance of Congenital Anomalies. EUROCAT guide 1.4 and reference documents . http://www.eurocat-network.eu/content/EUROCAT-Guide-1.4-Full-Guide.pdf. Published 2013. Updated 2013. Accessed 09/09, 2017.

56. Scholtens S, Smidt N, Swertz MA, et al. Cohort profile: LifeLines, a three-generation cohort study and biobank. Int J Epidemiol. 2015;44(4):1172-1180.

57. Reefhuis J, Gilboa SM, Anderka M, et al. The national birth defects prevention study: A review of the methods. Birth Defects Res A Clin Mol Teratol. 2015;103(8):656-669.

58. Tielemans E, Heederik D, Burdorf A, et al. Assessment of occupational exposures in a general population: Comparison of different methods. Occup Environ Med. 1999;56(3):145-151.

59. International Labor Office. International standard classification of occupations. 1988.

60. Matheson MC, Benke G, Raven J, et al. Biological dust exposure in the workplace is a risk factor for chronic obstructive pulmonary disease. Thorax. 2005;60(8):645-651.

61. Kromhout H, Vermeulen R. Application of job-exposure matrices in studies of the general population: Some clues to their performance. Eur Respir Rev. 2001(11):80-90.

62. Brouwers MM, van Tongeren M, Hirst AA, Bretveld RW, Roeleveld N. Occupational exposure to potential endocrine disruptors: Further development of a job exposure matrix. Occup Environ Med. 2009;66(9):607-614.

63. Van Tongeren M, Nieuwenhuijsen MJ, Gardiner K, et al. A job-exposure matrix for potential endocrine-disrupting chemicals developed for a study into the association between maternal occupational exposure and hypospadias. Ann Occup Hyg. 2002;46(5):465-477.

64. Rocheleau CM, Lawson CC, Waters MA, et al. Inter-rater reliability of assessed prenatal maternal occupational exposures to solvents, polycyclic aromatic hydrocarbons, and heavy metals. J Occup Environ Hyg. 2011;8(12):718-728.

65. de Vocht F, Zock JP, Kromhout H, et al. Comparison of self-reported occupational exposure with a job exposure matrix in an international community-based study on asthma. Am J Ind Med. 2005;47(5):434-442.

66. Peters S, Vermeulen R, Cassidy A, et al. Comparison of exposure assessment methods for occupational carcinogens in a multi-centre lung cancer case-control study. Occup Environ Med. 2011;68(2):148-153.

67. Schoenwolf GC, Brauer PR, Bleyl SB, Francis-West PH. Larsen's human embryology. 5th Revised edition ed. Elsevier - Health Sciences Division; 2014.

CHAPTER 2

Congenital anomalies in the offspring of occupationally exposed mothers: a systematic review and meta-analysis of studies using expert assessment for occupational exposures

Nynke Spinder
Jelmer R. Prins
Jorieke E.H. Bergman
Nynke Smidt
Hans Kromhout
H. Marike Boezen
Hermien E.K. de Walle

Human Reproduction. 2019;34(5):903–919.

ABSTRACT

Study question Is there an association between maternal occupational exposure to solvents, pesticides and metals as assessed by expert-based assessment and congenital anomalies in the offspring?

Summary answer There is an association between maternal occupational exposure to solvents and congenital anomalies in the offspring, including neural tube defects, congenital heart defects and orofacial clefts.

What is known already One important environmental risk factor for development of congenital anomalies is maternal occupational exposure to chemicals in the workplace prior to and during pregnancy. A number of studies have assessed the association with often conflicting results, possibly due to different occupational exposure assessing methods.

Study design, size, duration For this systematic review with meta-analysis, the search terms included maternal occupation, exposure, congenital anomalies and offspring. Electronic databases MEDLINE and EMBASE were searched for English studies up to October 2017.

Participants/materials, setting, methods Two reviewers independently screened all citations identified by the search. Case-control studies and cohort studies were included if (I) they reported on the association between maternal occupational exposure to solvents, pesticides or metals and congenital anomalies, and (II) assessment of occupational exposure was performed by experts. Data on study characteristics, confounders and odds ratios (ORs) were extracted from the included studies for four subgroups of congenital anomalies. Methodological quality was assessed using the Newcastle-Ottawa Scale. In the meta-analysis, random effects models were used to pool estimates.

Main results and the role of chance In total, 2806 titles and abstracts and 176 full text papers were screened. Finally, 28 studies met the selection criteria, and 27 studies could be included in the meta-analysis. Our meta-analysis showed that maternal occupational exposure to solvents was associated with neural tube defects (OR 1.51, 95%CI 1.09-2.09) and congenital heart defects (OR 1.31, 95%CI 1.06–1.63) in the offspring. Also maternal occupational exposure to glycol ethers, a subgroup of solvents, was associated with neural tube defects (OR 1.93, 95%CI 1.17-3.18) and orofacial clefts (OR 1.95, 95%CI 1.38–2.75) in the offspring. Only one study investigated the association between maternal occupational

exposure to solvents and hypospadias and found an association (OR 3.63, 95%CI 1.94-7.17). Results of the included studies were consistent. In our meta-analysis, we found no associations between occupational exposure to pesticides or metals and congenital anomalies in the offspring.

Limitations, reasons for caution A limited number of studies was included, which made it impossible to calculate pooled estimates for all congenital anomalies, analyse individual chemicals or calculate exposure-response relations. Bias could have been introduced because not all included studies corrected for potentially confounding factors.

Wider implications of the findings Employers and female employees should be aware of the possible teratogenic effects of solvent exposure at the workplace. Therefore, is it important that clinicians and occupational health specialist provide women with preconception advice on occupational solvent exposure, to reduce the congenital anomaly risk.

INTRODUCTION

Around 2-3% of pregnancies in Europe are affected by a major congenital anomaly [1]. The aetiology of most congenital anomalies is not fully understood, but genetic factors as well as environmental factors are involved. To decrease the prevalence of congenital anomalies, it is important to identify modifiable environmental factors and prevent maternal exposure to harmful factors. Examples of environmental factors known to increase the risk of having a child with a congenital anomaly include smoking during pregnancy [2] and increased body mass index (BMI) [2,3]. Air pollution is another factor that has been associated with development of congenital anomalies, in particular with congenital heart defects [4].

One important environmental factor that has been associated with development of congenital anomalies is maternal exposure to chemicals in the workplace prior to and during pregnancy. Most studies that have investigated maternal occupational exposure have focused on exposure to solvents, pesticides and metals. Exposure to these chemical substances have been associated with various adverse reproductive outcomes. For instance, occupational exposure to solvents has been associated with reduced fertility and increased risks of spontaneous abortion and congenital anomalies [5,6]. Pesticide and metal exposure in the workplace have been suggested to interfere with reproductive function and have been associated with prolonged time to pregnancy, spontaneous abortions, congenital anomalies, prematurity and reduced birth weight [5-8].

Epidemiological studies that have investigated the association between maternal occupational exposure and congenital anomalies in the offspring have conflicting results. One explanation for these divergent results may be the type of exposure assessment used, e.g. job title as proxy for exposure, self-reported exposure or expert-based assessment. Job title as proxy for exposure can introduce non-differential misclassification [8]. An example of using job title as proxy for exposure are studies reporting on the association between a specific occupational group (e.g. agricultural workers) and congenital anomalies in the offspring in which it is hypothesised that the congenital anomalies could be associated with an occupational exposure that is expected to be present in this occupation (e.g. pesticide exposure in agricultural workers). Using self-reported occupational exposure can introduce misclassification of exposure compared to expert assessment [9]. Both assessment methods may overestimate the effects of maternal occupational exposure and congenital anomalies in the offspring [8,9]. In this systematic review, we have therefore only included papers that used expert assessment in order to have less heterogeneous human evidence. Experts have, by training, a better understanding of the mechanisms of exposure [9] and know which agents and which levels of exposure play a role in specific jobs [10]. We considered both case-by-case expert assessment and Job-Exposure Matrices (JEMs) as expert-based assessments. Job-exposure matrices are occupational exposure assessment tools based on cross tabulations of jobs against occupational exposures where probability and intensity have been scored by exposure experts (occupational hygienists) [11]. Occupational hygienists assess occupational exposure on the individual level, whereas JEMs assign exposures at the job level.

The aim of this review is to summarise the current evidence about maternal occupational exposure to solvents, pesticides and metals and congenital anomalies in the offspring by conducting a systematic review and meta-analysis using expert assessment for occupational exposures.

MATERIALS AND METHODS

This systematic review was conducted using the methods of the Cochrane Collaboration [12] and reporting according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) statement [13]. The protocol of our systematic review is registered in PROSPERO, an International prospective register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42017053943).

Eligibility criteria, information sources, search strategy

A literature search of the electronic databases MEDLINE and EMBASE was conducted on 12 January 2017. Search strings included the indexing terms (MeSH terms, Emtrees and key terms): maternal occupation, exposure, congenital anomalies and offspring (Supplementary File 1). A search update was conducted on 23 October 2017.

Study selection

Case-control and cohort studies with a non-exposed control group were included if they reported on the association between maternal occupational exposure to solvents, pesticides or metals and subtypes of congenital anomalies in their offspring. Occupational exposure had to be assigned by an occupational exposure expert, through a JEM or by using expert literature, for example National Institute for Occupational Safety and Health criteria documents. Studies using occupation as a proxy for occupational exposure without involvement of occupational expertise and studies using self-reported exposure were excluded.

Congenital anomalies had to be diagnosed or reported by a medical expert, identified by birth (defect) registries or identified using established guidelines (e.g. International Classification of Disease(ICD)-codes, EUROCAT guidelines). Studies in which only the parents reported on the congenital anomalies were excluded. Only full text studies published in English, German, French and Dutch were included. Case-reports and reviews were excluded.

Data extraction

All identified hits were screened on title and abstract for eligibility by two reviewers (NSp and JP) independently. Full texts of all potentially eligible articles were screened for final selection by the same reviewers. The reference lists of all included articles and relevant reviews were also screened to identify further eligible studies. Disagreements between the two reviewers' assessments were resolved in consensus meetings. In case of persistent disagreement, a final decision was made by a third reviewer (HdW).

Data on study design, study population, study period, exposure, exposure assessment, outcome, outcome assessment, confounders and crude or adjusted odds ratios (OR) was extracted from the included studies. When certain information/data was missing, we contacted the corresponding author. One reviewer (NSp) extracted all of the data and

a second (JP) and third reviewer (JB, HdW, NSm, each one third of the extracted data) checked all of the extracted data.

Methodological quality

The quality of the studies was assessed by two reviewers independently (NSp and JP) using the Newcastle-Ottawa Scale, adjusted to study specific requirements, which is designed for assessing the quality of non-randomised studies in meta-analyses [14] (Supplementary File 2 and 3). 'Stars' could be awarded on different methodological quality items. A maximum of nine 'stars' could be allocated to each study. Although papers might have referred to methods papers, only index papers were used to assess methodological quality. Disagreements were discussed and resolved in consensus meetings between the first two reviewers (NSp and JP). To evaluate the inter-agreement of the methodological quality of the studies, we calculated the overall percentage agreement and Cohen's kappa a measure of congruence corrected for chance agreement [12].

Data synthesis

Meta-analyses were performed for the following categories of congenital anomalies: (I) neural tube defects, (II) congenital heart defects, (III) orofacial clefts and (IV) hypospadias, because these categories of major congenital anomalies are the most prevalent. Subgroup analyses were performed on cleft lip, with or without cleft palate, and cleft palate. Separate analyses were performed for the most prevalent subgroups of maternal occupational exposure to (a) solvents, (b), pesticides and (c) metals. A subgroup analysis was performed for maternal occupational exposure to glycol ethers, because this is a large subcategory of solvents.

The OR was used to calculate a pooled estimate. To reduce potential confounding effects, adjusted ORs were used for the meta-analyses where possible. When crude or adjusted ORs were not given, the available raw data was used in a 2x2 table to calculate the OR. When occupational exposure was categorised, categories were dichotomised so that the lowest category (no exposure) was tested against all other categories combined (e.g. low and high). Papers reporting zero exposed cases/controls were excluded from the meta-analysis because an OR could not be calculated. When multiple papers were based on the same study population, we selected a paper based on the following criteria: (I) results reported an estimate useful for the meta-analysis and (II) largest sample size.

A random effects method was used to pool effect estimates. Heterogeneity was examined by the I^2 index. If the I^2 index was higher than 50% [12], the results of the studies in the pooled analyses were considered to be heterogeneous, and no pooled estimate was calculated [12,15]. Sources of heterogeneity were explored by conducting subgroup analyses for differences in study design (cohort versus case-control studies), study population (case ascertainment by hospital versus registry), exposure time window (first trimester versus three months before conception through the first trimester), exposure assessment (industrial hygienist versus JEM), and methodological quality (per item) as assigned by the Newcastle-Ottawa Scale.

Publication bias was assessed by constructing funnel plots for the relation between various occupational exposures and congenital anomalies. Asymmetry of the funnel plots was assessed by Egger's test. If the *P*-value was <0.10, publication bias is likely [12,16]. All statistical analyses were performed with Comprehensive Meta-Analyses (version 3).

RESULTS

Study selection

In total, 2806 titles and abstracts were screened and 176 full texts were read (Figure 1). Screening the references of the included studies and other relevant reviews identified one additional eligible article. An updated search performed in October 2017 included one additional article. In total, 28 studies were included in the systematic review and 27 were included in the meta-analysis. One study was excluded from the meta-analysis because the results were based on the same study population as another included study.

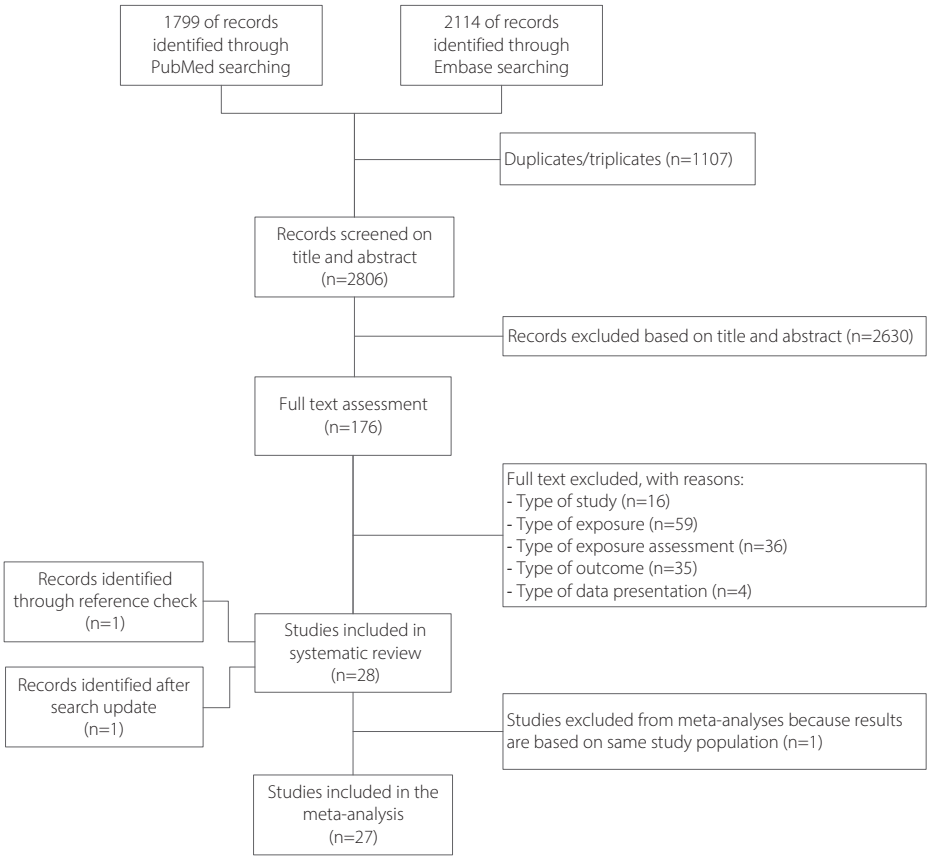


Figure 1 | Flowchart of study selection

Study characteristics

Table 1 shows the characteristics of the included studies, consisting of 26 case-control studies and two cohort studies. The included studies were conducted between 1980 and 2014. Most studies used birth registries or birth defect registries to identify children with congenital anomalies (n=16). Other studies were conducted in hospitals, rehabilitations centers, paediatric services and obstetric clinics. The critical time window of exposure was most often defined as three to one month before conception through the first trimester of pregnancy. Most studies used occupational hygienists to assess occupational exposure (n=15), whereas eleven studies used a JEM and two studies used expert based literature. In most studies, congenital anomalies were reported to registries by health care professionals, often by a clinical geneticist. When a study was performed in a hospital, diagnoses were confirmed by (paediatric) specialists. Most studies excluded cases diagnosed with chromosomal abnormalities or monogenic syndromes (Supplementary Table 1).

Table 1 | Study Characteristics of 28 Included Studies in the Systematic Review.

Study	Country	Study design	Study period	Source of case	Source of control	Exposure	Exposure time window	Method of occupational exposure assessment	Type of congenital anomalies	Identification method of congenital anomalies	Adjusted, matched or crude data	Adjustment for covariates	Risk of bias (NOS score)
Blatter et al. (1996)	The Netherlands	Case-control	1980-1992	Seven hospitals and two rehabilitation centers	Most from general population recruited from birth registries, some from seven hospital and two rehabilitation centers, all without congenital anomaly	Organic solvents Pesticides Mercury	Two weeks before conception until six weeks after conception	Expert assessed occupation, occupational task and rated exposure level. Occupational information was provided by mothers during a specific personal interview	Spina bifida aperta	Medical records were searched to identify spina bifida aperta cases	Stratified Adjusted [a]	Size of municipality and geographical location Vitamin A, anti-epileptics, ovulation stimulating agents, oral contraceptives, alcohol, smoking, positive family history of NTDs, consanguinity, diabetes, diagnosis of homocysteinaemia, parity, fetal loss	8
Brender et al. (2002)	USA	Case-control	1995-2000	Mexican Americans in the Texas NTD Project	Hospital or midwife-attended birthing center during the same time period as the case women	Solvents (including glycol ethers) * Pesticides Lead *	Three months before through three months after conception	Occupational codes were linked to specific exposures based on different literature sources. Occupational information was provided by mothers during an interview	NTD	Active surveillance of NTD births through multiple sources, including hospitals, birth centers, genetic clinics.	Matched Adjusted	Year of index birth and site of delivery Mother's age, education and BMI	8
Brender et al. (2006)	USA	Case-control	1995-2000	Mexican Americans in the Texas NTD Project	Hospital or midwife-attended birthing center during the same time period as the case women	Heavy metals (arsenic, cadmium, lead, mercury)	Three months before through three months after conception	Occupational codes were linked to specific exposures based on different literature sources. Occupational information was provided by mothers during an interview	NTD	Active surveillance of NTD birth through multiple sources, including hospitals, birth centers, genetic clinics.	Crude		6

Table 1. Continued

Study	Country	Study design	Study period	Source of case	Source of control	Exposure	Exposure time window	Method of occupational exposure assessment	Type of congenital anomalies	Identification method of congenital anomalies	Adjusted, matched or crude data	Adjustment for covariates	Risk of bias (NOS score)
Carbone et al. (2006)	Italy	Case-control	1998-2002	Paediatric service in highly agricultural district	Controls born in the same year in same municipality selected from public paediatric records	Pesticides	Before or during pregnancy	Directly asked by researchers/ experts during interviews	Hypospadias	Recorded in the paediatric service records and confirmed by surgical consultants	Adjusted [b]	Birth weight, parity, mother's age, mother's education, time to pregnancy, condom use, mother's gynaecological diseases, father's urogenital diseases, use of anti-abortion drugs, mother's alcohol use during pregnancy, same exposure variable of the other parent	8
Chevrier et al. (2006)	France	Case-control	1998-2001	Seven hospitals	Same hospitals as cases, but hospitalised for treatment of other disorder (infection, minor surgery)	Organic solvents	First trimester	Expert chemist assessed exposure using mothers work and job tasks provided by mothers during an interview in the hospital with a standardised questionnaire	Non-syndromic oral clefts	During initial hospitalization for surgery in the maxillofacial surgery department	Matched Adjusted [b]	Sex, age, mother's geographic origin and residence Study center, child's sex, mother's geographic origin	9
Cordier et al. (1992)	France	Case-referent	1984-1987	15 maternity hospitals	First infant born without anomaly after case child in same maternity hospital	Solvents	During pregnancy	Occupational histories of mothers, provided by mothers during an interview, were reviewed by an industrial hygienist	CHD Oral clefts	Cases were identified in hospital according to specific 'British Paediatric Association Classification of Diseases' codes	Matched Adjusted	Hospital of birth Residential area, age, and socioeconomic status of the mother	8

Table 1. Continued

Study	Country	Study design	Study period	Source of case	Source of control	Exposure	Exposure time window	Method of occupational exposure assessment	Type of congenital anomalies	Identification method of congenital anomalies	Adjusted, matched or crude data	Adjustment for covariates	Risk of bias (NOS score)
Cordier et al. (1997)	France, Italy, United Kingdom, the Netherlands	Case-control	1989-1992	Six EUROCAT registries	First infant born without anomaly after case child in same maternity hospital	Glycol ethers	First trimester	An expert chemist assessed exposure guided by a detailed description of women's occupational tasks provided by mothers during an interview	NTD CHD Oral clefts	Active case-finding by physicians, midwives, with help of hospital or registry staff following EUROCAT guidelines	Matched Adjusted	Place of birth, date of birth, mother's residence. Maternal age, socioeconomic status, area of residence, country of origin, and center	8
Cordier et al. (2001)	Slovakia	Case-control	1995-1996	26 maternity hospitals and obstetrical clinics	First infant born without anomaly after case child in same maternity hospital or clinic	Glycol ethers	First trimester	Chemist specializing in glycol ethers evaluated exposure using job description provided by mothers during an interview by their physicians using a study questionnaire	NTD CHD Oral clefts	No description	Adjusted [c]	Maternal age at birth, socioeconomic status and residence	4
Desrosiers et al. (2012)	USA	Case-control	1997-2002	National Birth Defects Prevention Study	Non-malformed live birth selected from birth certificates or hospital records from the same base population as the cases	Organic solvents	One month before through end of third month of pregnancy	Occupational epidemiologists and industrial hygienists rated maternal jobs provided by mothers during a telephone interview	NTD Oral clefts	Surveillance by birth defect registries, clinical geneticists performed review of medical records to confirm eligibility	Adjusted [b]	Maternal age, race/ethnicity, education, pre-pregnancy BMI, folic acid and smoking	9
Garlantézec et al. (2009)	France	Prospective cohort	2002-2005	Recruitment by gynaecologists, obstetricians or ultrasono-graphers at visits for prenatal care	Recruitment by gynaecologists, obstetricians or ultrasonographers at visits for prenatal care	Solvents	Occupation before 19 weeks of gestational age	JEM based on occupation code and industrial activity code based on information provided by a questionnaire before 19 weeks of gestation	CHD Oral clefts	Validation of anomaly by a paediatrician based on clinical examination of live-born infants, pathology and karyotype examinations on non-live births	Adjusted	Alcohol consumption Maternal age, tobacco and alcohol consumption, education level	8

Table 1. Continued

Study	Country	Study design	Study period	Source of case	Source of control	Exposure	Exposure time window	Method of occupational exposure assessment	Type of congenital anomalies	Identification method of congenital anomalies	Adjusted, matched or crude data	Adjustment for covariates	Risk of bias (NOS score)
Gilboa et al. (2012)	USA	Case-control	1997-2002	National Birth Defects Prevention Study	Non-malformed live birth selected from birth certificates or hospital records	Organic solvents	One month before through end of first trimester	Industrial hygienists rated maternal jobs based on job description provided by mother from an interview [d]	Isolated CHD	Surveillance by birth defect registries, clinical geneticists performed review of medical records to confirm eligibility	Adjusted	Maternal age, race/ethnicity, education, smoking, periconceptional folic acid intake	8
Giordano et al. (2010)	Italy	Case-control	2005-2007	Two Roman hospitals	Healthy male infants attending the Outpatient Vaccination Service	Pesticides * Heavy metals	Three months before through three months after conception	JEM using job title provided during an interview	Hypospadias	Recruited if required surgical treatment (1st, 2nd and 3th degree hypospadias)	Adjusted	BMI at conception, education father	8
Jackson et al. (2004)	USA	Case-control	1981-1989	Hospitals	Infant born without cardiovascular malformations in same hospital	Lead	Three months before conception through first trimester	Industrial hygienists and occupational epidemiologists reviewed all jobs, a JEM and self-reported exposure was used and reviewed by staff having expertise. Mother was classified as exposed if classified exposed by any of the methods.	Total anomalous pulmonary venous return (TAPVR) (CHD)	Confirmed by echocardiography, cardiac catheterization, surgery, and/or autopsy. Updated at one year of age.	Stratified	Stratified by month, year, and hospital of birth	5
Kalfa et al. (2015)	France	Case-control	2009-2014	Multi-institutional/ hospitals	Hospitalized boys without congenital malformation	Organic solvents Pesticides	During all three trimesters of pregnancy	JEM using occupational information from a questionnaire filled in by surgeon or endocrinologist	Isolated hypospadias	Clinical diagnosis made via direct clinical examination by a paediatric urologist and/ or paediatric endocrinologist	Matched	Ethnic origin	4

Table 1. Continued

Study	Country	Study design	Study period	Source of case	Source of control	Exposure	Exposure time window	Method of occupational exposure assessment	Type of congenital anomalies	Identification method of congenital anomalies	Adjusted, matched or crude data	Adjustment for covariates	Risk of bias (NOS score)
Lorente et al. (2000)	France, Italy, United Kingdom, Netherlands	Case-referent	1989-1992	Six EUROCAT registrations	First infant born without anomaly on same date or in same town or next born infant	Glycol ethers * Lead	First trimester	Industrial hygienist based on job description provided by mother during an interview	Oral clefts	Cases were identified by the registries	Adjusted [e]	Center, mothers socioeconomic status, urbanization, country of origin, maternal age	8
Makelarski et al. (2014)	USA	Case-control	1997-2002	National Birth Defects Prevention Study	Non-malformed live births selected from birth certificates or hospital records	Pesticides	One month before through two months after conception	Industrial hygienist using coded job information provided by mothers during a telephone interview	NTD	Surveillance by birth defect registries, clinical geneticists performed review of medical records to confirm eligibility	Adjusted	Maternal BMI (continuous), maternal education, study site	5
Morales-Suarez-Varela et al. (2011)	Denmark	Prospective cohort	1997-2002	Danish National Birth Cohort	All other male births from the Danish National Birth Cohort	Pesticides Heavy metals	Three months before pregnancy and during pregnancy	JEM using job title provided by women in a telephone interview at 16 weeks of gestation	Hypospadias	National Hospital Discharge Registry which included information about congenital anomalies based on the ICD10	Adjusted [b]	Parental age and smoking, earlier spontaneous abortion, parity, birth weight, gestational age, oral contraceptive use, treatment of infertility, time to conceive, maternal alcohol consumption, binge drinking, pre-pregnancy BMI, vegetarian diet, gynecological disease	8

Table 1. Continued

Study	Country	Study design	Study period	Source of case	Source of control	Exposure	Exposure time window	Method of occupational exposure assessment	Type of congenital anomalies	Identification method of congenital anomalies	Adjusted, matched or crude data	Adjustment for covariates	Risk of bias (NOS score)
Nassar et al. (2009)	Australia	Case-control	1980-2000	Western Australian Birth Defects Registry	Random sample from Western Australian Maternal and Child Health Research Database	Pesticides Heavy metals	At least 20 weeks or more gestation	Exposure assigned by researchers according to a JEM using occupation available from the Western Australian Maternal and Child Health Research Database	Hypospadias	Statutory and voluntary sources of notification coded with the ICD9	Matched Adjusted	Birth year Maternal age, parity, race, location, marital status, socioeconomic status, plurality, small for gestational age, year of birth	9
Pettigrew et al. (2016) *	USA	Case-control	1997-2002	National Birth Defects Prevention Study	Non-malformed live birth selected from birth certificates or hospital records	Pesticides	One month before through one month after conception	Industrial hygienist using coded job information provided by mothers during a telephone interview	Spina bifida	Surveillance by birth defect registries, clinical geneticists performed review of medical records to confirm eligibility	Adjusted	Maternal race/ ethnicity, maternal education level, study site	5
Pierik et al. (2004)	The Netherlands	Nested Case-control	1999-2001	Child health care centers Rotterdam	Boys without cryptorchidism or hypospadias if their age was compatible with the observed age range of cases from child health care centers Rotterdam	Pesticides	The year before delivery	JEM based on job title provided by parents in an interview	Hypospadias	Child health care center physician trained by paediatric urologist and paediatric endocrinologist	Crude		6

Table 1. Continued

Study	Country	Study design	Study period	Source of case	Source of control	Exposure	Exposure time window	Method of occupational exposure assessment	Type of congenital anomalies	Identification method of congenital anomalies	Adjusted, matched or crude data	Adjustment for covariates	Risk of bias (NOS score)
Rocheleau et al. (2011)	USA	Case-control	1997-2002	National Birth Defects Prevention Study	Non-malformed live birth selected from birth certificates or hospital records	Pesticides	One month before conception through end of first trimester	Exposures were assigned by an expert, assisted by a JEM, from a job history completed by mothers during a telephone interview	Hypospadias (second and third degree), categorized as isolated or multiple	Surveillance by birth defect registries, clinical geneticists performed review of medical records to confirm eligibility	Adjusted	All other pesticides, parity, maternal race and age, infant gestational age, study center	6
Rocheleau et al. (2015)	USA	Case-control	1997-2002	National Birth Defects Prevention Study	Non-malformed live birth selected from birth certificates or hospital records	Pesticides	One month before conception through end of first trimester	Exposure was assigned by an expert-guided task-exposure matrix and job history details reported by mothers during a telephone interview	CHD	Surveillance by birth defect registries, clinical geneticists performed review of medical records to confirm eligibility	Adjusted [b]	Maternal education, study site, income, pre-pregnancy BMI, alcohol consumption, language of interview, paternal education	8
Shaw et al. (1999)	USA	Case-control	1987-1988 [f]	California Birth Defects Monitoring Program	Randomly selected from infants born alive in same geographic area and time period without major congenital anomaly diagnosed before first birthday	Pesticides	One month before conception through end of first trimester	Industrial hygienist assigned exposure using narrative job information provided by mothers during a telephone interview	NTD Conotruncal heart defects Oral clefts (isolated)	Surveillance by birth defect registry. Determined by medical geneticist using detailed information	Adjusted [b]	Maternal periconceptional vitamin use, cigarette smoking, education level and race/ethnicity	6

Table 1. Continued

Study	Country	Study design	Study period	Source of case	Source of control	Exposure	Exposure time window	Method of occupational exposure assessment	Type of congenital anomalies	Identification method of congenital anomalies	Adjusted, matched or crude data	Adjustment for covariates	Risk of bias (NOS score)
Snijder et al. (2012)	The Netherlands	Case-control	2003-2010	HAVEN study	Healthy children with similar age to case children ascertained in regular health checks by child physicians in the same region	Pesticides Heavy metals	Four weeks prior to conception until eight weeks after conception	JEM using job title and description of work tasks provided by mothers in a questionnaire	CHD	Anomalies were identified with echocardiography and/or cardiac catheterization and/or surgery	Adjusted	Maternal age, educational level, ethnicity, parity, CHD in family, periconceptional alcohol use, periconceptional medication use, periconceptional folic acid use, urban density	8
Spinder et al. (2017)	The Netherlands	Case-control	1997-2013	EUROCAT Northern Netherlands	Malformed babies/foetuses registered in EUROCAT with a non-chromosomal/ non-monogenic disorder, without an oral cleft	Solvents Pesticides Heavy metals	Three months before conception through the first trimester	JEM using job title provided by mothers in a questionnaire	Isolated oral clefts	Surveillance by a birth defect registry. Classification of congenital anomalies is performed according to EUROCAT guidelines	Adjusted	Child sex and previous births	6
Tikkanen et al. (1988)	Finland	Case-control	1980-1981	Finnish Register of Congenital Malformations	Next born infant in same Maternity care District	Organic solvents Pesticides	First trimester	Industrial hygienist explored and grouped exposure information provided by mothers during an interview	CHD	Experienced pathologist checked diagnosis based on autopsy findings of stillbirths. Paediatric cardiologist identified through catheterization, echocardiography, cardiac surgery or clinical follow-up	Matched	Next born and same district	4

Table 1. Continued

Study	Country	Study design	Study period	Source of case	Source of control	Exposure	Exposure time window	Method of occupational exposure assessment	Type of congenital anomalies	Identification method of congenital anomalies	Adjusted, matched or crude data	Adjustment for covariates	Risk of bias (NOS score)
Vrijheid et al. (2002)	United Kingdom	Case-control	1980-1989/ 1992-1996	National Congenital Anomaly System	All cases with a congenital anomaly registered in the National Congenital Anomaly System	Pesticides Heavy metals	Job early in pregnancy	JEM based on job classified by industrial hygienists. Jobs were reported on standardised reporting forms collected from doctors and midwives	Hypospadias	Notification from doctors and midwives using standardized reporting forms	Adjusted	Year of birth, region, maternal age, social class of mother, social class of father	7
Wang et al. (2015)	China	Case-control	2012-2013	Two university medical centers	Healthy infants with similar age to case children from same medical centers	Pesticides Heavy metals	Four weeks prior to conception until end first trimester	JEM using job description provided by parents in a face to face interview	CHD (isolated)	Diagnosis confirmed by cardiac catheterization/ paediatric cardiologists	Adjusted	Maternal age at birth, maternal education level, gravity, parity, artificial abortion, folic acid use, medication use, drinking capacity, area of residence periconceptionally	7

NOS = Newcastle-Ottawa Scale, NTD = Neural Tube Defect, USA = United States of America, BMI = body mass index, CHD = Congenital Heart Defect, EUROCAT = European Registry Of Congenital Anomalies and Twins, JEM = Job Exposure Matrix, ICD = International Classification of Diseases. * = not included in the meta-analysis. [a] = crude odds ratios are shown because adjusted did not change results, [b] = raw data was used to calculate crude odds ratios for meta-analyses because subgroups of exposures were merged, [c] = raw data for NTD was used because odds ratios was not given, cleft palate without cleft lip were only adjusted for maternal age at birth and residence, [d] = exposure was assisted with a literature-based approach as well, for this study data of the expert consensus-based approach was used, [e] = raw data was used to calculate odds ratios for meta-analyses because subgroups of congenital anomalies were merged, [f] = study period 1987-1989 for oral clefts.

Risk of bias of included studies

The results of the methodological quality assessment of the included studies are presented in Supplementary Table 2. Study quality varied from poor (4 stars) to high (9 stars). All case-control studies met the quality criteria for same method of exposure ascertainment for cases and controls. Most of the case-control studies included met quality criteria for adequate case definition, selection of controls, and definition of controls. Seven case-control studies did not meet quality criteria on representativeness of the cases. Six case-control studies scored medium risk of bias on comparability of cases and controls based on the design or analysis, and eight studies scored a high risk of bias on this item. Six case-control studies did not meet criteria on ascertainment of exposure. Most case-control studies (n=17) did not report non-response rate, making it not possible to judge the likelihood of bias on this item (attrition bias).

The two cohort studies included in this systematic review met quality criteria on selection of the non-exposed part of the cohort, adequate ascertainment of exposure, demonstration that the outcome of interest was not present at start of study, comparability of cohort on the basis of design or analysis and ascertainment of exposure, and the follow-up was long enough for outcomes to occur. Garlantézec et al. did not meet the criteria on representativeness of the exposed cohort [17]. Morales-Suarez-Varela et al. did not meet the criteria on adequacy of follow up [18].

Agreement on methodological quality between the two reviewers was moderate (overall agreement 83% (238/288); Cohen's Kappa statistic: 0.45). Most disagreements were caused by criteria on comparability and ascertainment of exposure.

Synthesis of results

Table 2 shows an overview of the results of our meta-analyses. Results of individual studies are presented in Supplementary Table 1. Forest plots of significant findings of the main analyses are shown in the main figures. All other forest plots and all funnel plots are shown in the supplementary figures.

Table 2 | Overview of associations between maternal exposure and several congenital anomalies

Congenital anomaly	Maternal occupational exposure	Studies	Exposed/ total cases	Exposed/ total controls	Pooled OR	95% CI	Hetero-geneity (%)
Neural tube defects							
	Solvents	4	124/888	419/4145	1.51	1.09-2.09	35
	Glycol ethers	2	29/110	142/882	1.93	1.17-3.18	0
	Pesticides	4*	183/1097	918/3734	0.93	0.76-1.15	0
	Metals	2	12/458	18/539	NA	NA	82
Congenital heart defects							
	Solvents	6	185/2526	848/6744	1.31	1.06-1.63	0
	Glycol ethers	2	61/291	142/882	1.63	0.94-2.84	18
	Pesticides	5*	1088/4742	970/4477	0.81	0.54-1.21	38
	Metals	3	27/1185	48/1595	1.83	0.65-5.20	49.8
Orofacial clefts							
	Solvents	7*	354/1854	2111/11120	NA	NA	65
	Glycol ethers	3*	91/256	183/1037	1.95	1.38-2.75	0
	Pesticides	2	39/644	131/4773	NA	NA	57
	Metals	2	15/487	89/5107	1.62	0.91-2.86	0
Cleft lip with or without cleft palate							
	Solvents	5	198/866	1532/8371	1.35	1.10-1.66	8
	Glycol ethers	3	61/167	183/1037	1.95	1.38-2.75	0
	Pesticides	2	30/449	131/4773	1.30	0.84-2.01	0
	Metals	2	9/327	89/5107	1.45	0.70-3.01	0
Cleft palate							
	Solvents	5	142/966	1532/8371	1.25	0.94-1.65	26
	Glycol ethers	3*	30/89	183/1037	1.85	1.10-3.09	0
	Pesticides	2	9/195	131/4773	NA	NA	70
	Metals	2	6/160	89/5107	2.06	0.63-6.75	26
Hypospadias							
	Solvents	1	7/300	5/302	3.63[a]	1.94-7.17	
	Pesticides	7	227/5748	1190/82120	0.97	0.75-1.24	24
	Metals	4	89/4870	1303/79939	NA	NA	67

Bold values represent statistically significant values. * = Egger's test indicated that publication bias was likely, NA=not applicable: pooled estimate could not be calculated because of heterogeneity (>50%). [a]= no pooled OR, because only one study is included.

Neural tube defects

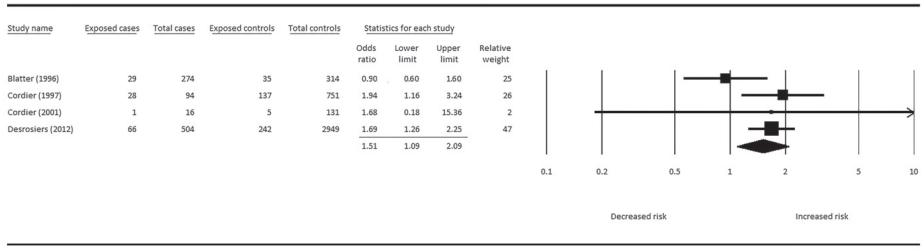
Five papers examined the association between occupational exposure to solvents and neural tube defects [19-23]. One study was excluded from the meta-analysis because the OR could not be calculated [20]. Two studies included in the meta-analysis reported a positive association between solvent exposure and neural tube defect [22,23]. The pooled estimate of the forest plot in Figure 2 showed that maternal occupational exposure to solvents was associated with a higher risk of neural tube defects in the offspring (OR 1.51, 95%CI 1.09-2.09). Egger's test indicated that publication bias was unlikely (Supplementary Figure 1). A subgroup analysis was performed on the three studies that reported on glycol ethers as exposure [20-22]. One study was excluded from the meta-analysis because the OR could

not be calculated [20]. The pooled estimate showed a statistically significant higher risk of neural tube defects in the offspring (OR 1.93, 95%CI 1.17-3.18, Supplementary Figure 2). The likelihood of publication bias could not be assessed, because only two studies were included.

Five studies assessed the relation between occupational exposure to pesticides and neural tube defects [19,20,24-26]. We excluded Pettigrew et al. from the meta-analysis because they used the same study population as Makelarski et al, and this last study had a larger sample size. No association was found between pesticide exposure and neural tube defects (OR 0.93, 95%CI 0.76-1.15, Supplementary Figure 3). Egger's test indicated that publication bias is likely (Supplementary Figure 4).

Three studies investigated the association between exposure to metals and neural tube defects [19,20,27]. Two studies retrieved the cases from the Texas Neural Tube Defect project [20,27]. We included Brender et al. (2006) in the meta-analysis because it assessed several classes of heavy metals compared to Brender et al. (2002), which only assessed maternal occupational exposure to lead. The study of Blatter et al. showed an association in the opposite direction between exposure to metals and neural tube defects. Because the results were heterogeneous, no pooled estimate could be calculated ($\chi 2 = 5.6$, df = 1, $P = 0.02$, $I^2 = 82\%$, Supplementary Figure 5). This heterogeneity and publication bias could not be assessed because only two studies are included.

Figure 2 | Forest plot of maternal occupational exposure to solvents and risk of neural tube defects in offspring.



Heterogeneity: $\chi 2 = 5.54$, df = 3, $P = 0.21$, $I^2 = 35\%$.

Congenital heart defects

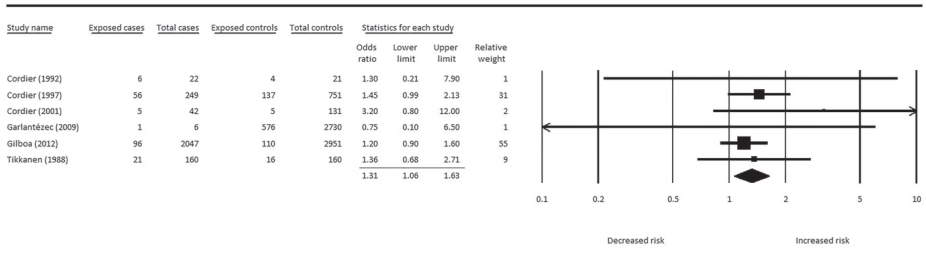
Six papers assessed the relation between occupational exposure to solvents and congenital heart defects in the offspring [17,21,22,28-30]. None of the studies in the meta-analysis found an association between exposure to solvents and congenital heart defects as a group. However, several studies found increased ORs for specific phenotypes of congenital heart

defects [21,22,29], and the forest plot in Figure 3 showed an association between maternal occupational exposure to solvents and congenital heart defects in the offspring (OR 1.31, 95%CI 1.06–1.63). Egger's test indicated that publication bias was unlikely (Supplementary Figure 6). A subgroup analysis was performed on two studies that reported on glycol ethers as exposure [21,22]. The pooled estimate of maternal occupational exposure to glycol ethers and congenital heart defects in the offspring showed no significant association (OR 1.63, 95%CI 0.94–2.84, Supplementary Figure 7). The likelihood of publication bias could not be assessed.

Five studies assessed the association between maternal occupational exposure to pesticides and congenital heart defects [26,30-33]. Shaw et al. (1999) included only cases with conotruncal congenital heart defects [26]. None of the studies showed an increased OR. The pooled estimate showed no association between mothers who were occupationally exposed to pesticides and congenital heart defects in the offspring (OR 0.81, 95%CI 0.54 – 1.21, Supplementary Figure 8). Egger's test indicated publication bias is likely (Supplementary Figure 9).

Three studies assessed the relation between exposure to metals and congenital heart defects [32-34]. Jackson et al. (2004) only included cases with one specific congenital heart disease: total anomalous pulmonary venous return [34]. Only the study of Wang et al. showed an association between occupational exposure to metals and congenital heart defects in the offspring [33]. The pooled estimate showed no significant association (OR 1.83, 95%CI 0.65–5.20, Supplementary Figure 10). Egger's test indicated that publication bias is unlikely (Supplementary Figure 11).

Figure 3 | Forest plot of maternal occupational exposure to solvents and risk of congenital heart defects in offspring



Heterogeneity: $\chi^2 = 2.58$, df = 5, $P = 0.76$, $I^2 = 0\%$

Orofacial clefts

Eight studies investigated the association between maternal occupational exposure to solvents and oral clefts in the offspring [17,21-23,28,35-37]. We excluded Lorente et al. from the meta-analysis because they used the same study population as Cordier et al. (1997). Cordier et al. (1997) included all solvent subclasses whereas Lorente et al. only studied exposure to glycol ethers. Three studies reported a positive association between solvent exposure and oral clefts in the offspring [17,22,35]. These results were too heterogeneous to calculate a pooled estimate ($\chi^2 = 17.3$, df = 6, P = 0.01, $I^2 = 65\%$) and the source of this heterogeneity could not be explored (Supplementary Figure 12). Egger's test indicated publication bias was likely (Supplementary Figure 13). We performed a subgroup analysis on data from five studies that reported separately on cases with cleft lip with or without cleft palate and cleft palate [21-23,35,37]. The studies of Chevrier et al. and Cordier et al (1997) concluded that there was an association between exposure to solvents and cleft lip with or without cleft palate. The pooled estimate in our meta-analyses did show an association as well (OR 1.35, 95%CI 1.10–1.66, Supplementary Figure 14). Egger's test indicated publication bias was unlikely (Supplementary Figure 15). None of the studies reporting on the exposure to solvents and cleft palate in offspring did show an association, nor did the pooled estimate show a significant association (OR 1.25, 95%CI 0.94–1.65, Supplementary Figure 16). Egger's test indicated publication bias was unlikely (Supplementary Figure 17).

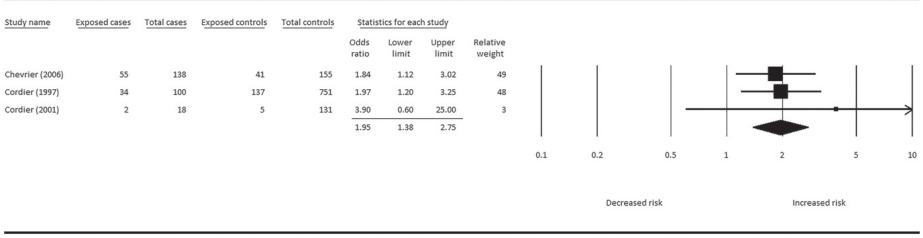
Furthermore, we performed subgroup analyses on three studies that reported on glycol ethers, a subgroup of solvents [21,22,35]. The pooled estimate of maternal occupational exposure to glycol ethers showed an association with orofacial clefts in the offspring (OR 1.95, 95%CI 1.38–2.75, Figure 4). Publication bias was likely (Supplementary Figure 18). Additionally, separate analyses on cleft lip with or without cleft palate and cleft palate alone with these same studies were performed. Both analyses showed an association when mothers are occupationally exposed to glycol ethers (OR 1.95, 95%CI 1.31–2.92; OR 1.85, 95%CI 1.10–3.05, respectively) (Supplementary Figure 19 and 21). Egger's test indicated publication bias was unlikely for cleft lip with or without cleft palate, and likely for cleft palate alone (Supplementary Figure 20 and 22).

Two studies assessed the association between maternal occupational exposure to pesticides and oral clefts in the offspring [26,37]. Only the study of Spinder et al. found a positive association. The results were too heterogeneous to calculate a pooled estimate (χ^2=2.3, df=1, P=0.13, I^2=57%, Supplementary Figure 23). Heterogeneity and publication bias could not be assessed, because only two studies were included. When a separate

analysis on cleft lip with or without cleft palate was performed the pooled estimate with these two studies estimate showed no significant association (OR 1.30, 95%CI 0.84–2.01, Supplementary Figure 24). The results for cleft palate were too heterogeneous to calculate a pooled estimate (χ^2 =3.4, df = 1, P = 0.07, I^2 = 70%, Supplementary Figure 25). The source of this heterogeneity could not be assessed because only two studies were included.

Two studies assessed the relation between exposure to metals and oral clefts [36,37]. The pooled estimate showed no significant association between occupational exposure to metals and oral clefts in the offspring (OR 1.62, 95%CI 0.91–2.86, Supplementary Figure 26). Publication bias could not be assessed, because only two studies were included. When a separate analysis on cleft lip with or without cleft palate and cleft palate alone was performed with these two studies, the pooled estimate showed no significant association (OR 1.45, 95%CI 0.70–3.01; OR 2.06, 95%CI 0.63–6.75, respectively) (Supplementary Figure 27 and 28).

Figure 4 | Forest plot of maternal occupational exposure to glycol ethers and risk of oral clefts in offspring



Heterogeneity: χ^2 = 0.58, df = 2, P = 0.75, I^2 = 0%

Hypospadias

Only one study assessed the association between maternal occupational exposure to solvents and hypospadias in the offspring [38]. This study found an association between exposure to solvents and hypospadias in the offspring (OR 3.63, 95%CI 1.94-7.17).

Eight studies assessed the association between maternal occupational exposure to pesticides and hypospadias [18,38-44]. We excluded one study from the meta-analysis because an OR could not be calculated due to zero exposed mothers in the control group [40]. Only the study of Kalfa et al. showed an association between exposure to pesticides and hypospadias [38]. The pooled estimate showed no association (OR 0.97, 95%CI 0.75-1.24, Supplementary Figure 29). Egger's test indicated publication bias is unlikely (Supplementary Figure 30).

Four studies assessed the association between maternal exposure to metals and hypospadias [18,40,41,44]. Only one of these studies showed an increased risk when mothers were occupationally exposed to metals [41]. The results were heterogeneous ($\chi^2 = 9.20$, df = 3, $P = 0.03$, $I^2 = 67\%$, Supplementary Figure 31), which meant that no pooled estimate could be calculated. The heterogeneity in results between studies could be explained by differences in recruitment of cases. Giordano et al. recruited children with a congenital anomaly at the hospital while the other studies retrieved their cases from registries [40]. The heterogeneity in results might also be explained by variations in methodological quality. One study scored high in risk of bias on control definition because there was no definition of controls stated [44]. Three studies had high risk of bias because the non-response rate between cases and controls was either not described or not comparable [18,40,44]. Egger's test indicated publication bias was unlikely (Supplementary Figure 32).

DISCUSSION

Main findings

The aim of this systematic review and meta-analysis was to summarise the current evidence about maternal occupational exposure and congenital anomalies in the offspring. Our meta-analysis showed that maternal occupational exposure to solvents is positively associated with neural tube defects in the offspring, especially exposure to glycol ethers. Maternal occupational exposure to solvents also appeared to be positively associated with congenital heart anomalies in the offspring. Furthermore, we found an association between an increased risk of orofacial clefts in the offspring and maternal occupational exposure to glycol ethers. This was also seen for cleft lip with or without cleft palate and cleft palate alone. Hypospadias in the offspring was also positively associated with maternal exposure to solvents, however this result was only based on one study. For maternal exposure to pesticides and metals no evidence for an association was found for the congenital anomalies considered.

Strengths and limitations

Our study has several strengths. This is the first review that has summarised and evaluated literature of both different subtypes of congenital anomalies and different subtypes of occupational exposures. Another strength of this review is that we used strict criteria on the definition of congenital anomalies. We used EUROCAT guidelines and definitions for major congenital anomalies because of their reliability [45]. EUROCAT has been registering congenital anomalies since 1979 and has strict inclusion criteria for major congenital anomalies. Furthermore, we included studies that used ICD codes for inclusion of congenital anomalies. Most studies included in our review retrieved case information from birth registries and birth defect registries. Those studies used EUROCAT guidelines or ICD codes as inclusion criteria for congenital anomalies. Other studies used hospital charts or diagnoses by medical experts. Particular birth defects may have been included in some studies and excluded from other studies depending upon which classification method was used. From the study of Hansen et al., it is known that this results in similar estimates of birth defect risks [46]. Parental self-reporting can introduce misclassification of congenital anomalies because of low reliability due to low recognition and recall bias of the anomaly [47], which is why we excluded studies that used parental reporting on congenital anomalies. Another strength is that we have only included studies that used expert assessment for defining occupational exposures or expert judgement, as the basis for assignment at the

job level, via a JEM. Studies included in other reviews often used self-reported occupational exposure for exposure assessment or job title as a proxy of occupational exposure. Self-reported occupational exposure can introduce misclassification of exposure [9]. Using job description as proxy for exposure can introduce non-differential misclassification [8]. Occupational hygienists assess occupational exposure on an individual level, whereas JEMs designed by experts can describe exposures on a group level. Studies using those methods reduce the risk of recall bias and differential misclassification of exposure compared to studies based on self-reported exposure [48,49]. Furthermore, a strength of our review is that most included studies in this systematic review used an adequate exposure time window. This is important, because the critical period for the development of most congenital anomalies is the first month before conception until the end of the first trimester. During the month before conception, maternal oocytes are vulnerable to chemical exposure. In the first trimester after conception, chemical exposure can affect the developing embryo. After this period, organogenesis is completed and the foetus is less vulnerable to chemical exposure for developing most congenital anomalies [47]. Finally, a strength of this review is that only includes studies reporting on major congenital anomalies. Studies reporting minor congenital anomalies were excluded because they have fewer medical, functional, societal and cosmetic consequences, and the definitions, diagnoses and reporting of minor anomalies are very variable [45]. Additionally, several studies have combined all major congenital anomalies in their analysis. Aetiology differs between congenital anomalies of different organ systems, which makes combining congenital anomalies of different organ origins unrealistic and analysis meaningless. For this reason, we excluded studies that did not report on congenital anomalies in separate categories.

We had to group birth defects by anatomical region. This could have been a limitation for congenital heart defects in particular. This review shows a positive association between occupational exposure to solvents and congenital heart defects but, because congenital heart defects are a heterogeneous group of birth defects, it is possible that this association is true for some types of heart defects and not for others. Also we did not find an association between occupational exposure to pesticides or metals and congenital heart defects overall, however it is still possible that specific types of heart defects might have been associated with these exposures. Our study has also some other limitations. It is possible that we missed relevant publications. Our original search was performed in January 2017, with an additional search performed in October 2017 that identified one additional study [37]. During further preparation of the manuscript, we carefully have tracked

publications in the field of this systematic review. Another limitation is that it was not possible to calculate pooled estimates for some specific congenital anomalies because too few included studies reported on the congenital anomaly or the occupational exposure. Furthermore, it is a limitation that it was not possible to analyse individual chemicals, we examined only generic occupational exposure classes in this review. It was also not possible to study exposure-response relations as not all included studies reported levels of exposure. Even when studies did report on level of exposure, it is questionable whether categories of exposure are comparable between studies because studies do not handle strict criteria for categorising levels of exposure. Dichotomising exposure could have masked the effect of a specific exposure on the development of congenital anomalies. Some studies found associations only at high doses, but not for 'any exposure' [31,35]. Those studies were included in our meta-analysis with the non-significant 'any exposure' OR. Another limitation is that little is known about the association between occupational exposure and multiple congenital anomalies (i.e. major congenital anomalies in more than one organ system). It is possible that one occupational exposure contributes to anomalies in multiple organ systems. Furthermore, eight studies did not correct for any confounding factors such as maternal age, folic acid use or maternal education. Not correcting for confounding factors leads to a high risk of bias and may result in an overestimation of the effect of occupational exposure on the development of congenital anomalies in the offspring [50]. Finally, it is important to interpret the results with caution due to the likelihood of publication bias. Although Egger's test did not indicate the presence of publication bias in most meta-analyses, our funnel plots and Egger's tests are based on fewer than ten studies. It is known that Egger's test is more reliable when at least ten studies are included in the meta-analysis [12,51]. Furthermore, Egger's test did indicate that publication bias is likely in the meta-analysis on occupational exposure to pesticides and congenital heart defects. This could be a false positive finding, because all included studies are non-significant studies, which makes Egger's less reliable [12,51]. In addition, the positive Egger's test regarding the meta-analysis on occupational exposure to solvents and oral clefts could be a false positive finding, because the included studies were heterogeneous (I^2 >50%) [12,51].

Comparison with existing literature

Several earlier reviews have summarised the literature regarding occupational exposure and congenital anomalies in offspring. In particular, two meta-analyses have been performed on the association between maternal occupational pesticide exposure and congenital anomalies [52,53]. The first meta-analysis focused on children with hypospadias and found

that maternal occupational exposure to pesticides is not associated with hypospadias in the offspring, when only studies using JEMs were included (OR 0.93, 95%CI 0.24-3.65, based on two studies using a JEM) [52]. This result is in line with the results of our study, where we did not find an association between maternal occupational exposure to pesticides and hypospadias in the offspring (OR 0.87, 95%CI 0.73–1.05, based on seven studies). Both studies included in the review of Rocheleau were included in our review. We included an additional five studies assessing the association between maternal occupational pesticide exposure and hypospadias that were published since March 2008.

Another meta-analysis, Romitti *et al.* (2007), studied the association between maternal occupational pesticide exposure and oral clefts in the offspring [53]. They suggested that maternal occupational exposure to pesticides can lead to a modest increase in the risk of having a child with an oral cleft (OR 1.37, 95%CI 1.04-1.81). In our meta-analysis, we were unable to estimate a pooled OR, because the studies were too heterogeneous and we included only two papers. The difference between our review and Romitti et al. is that we were restricting our review to those studies with expert assessment of maternal occupational exposure.

Conclusions and Implications

Our meta-analysis included 27 studies, examining the association between maternal occupational exposure and congenital anomalies in the offspring, each of which used expert assessment to assess occupational exposure. We concluded that maternal occupational exposure to solvents is associated with an increased risk of neural tube defects, congenital heart anomalies and orofacial clefts in the offspring. Occupational health specialists, employers and female employees should be aware of the possible teratogenic effects of solvent exposure at the workplace. Clinicians should provide women with preconception advice on exposure to solvents at the workplace to prevent neutral tube defects, congenital heart defects and orofacial clefts. Further research should focus on specific chemicals, use expert-based exposure assessment, and perform dose-response evaluation.

REFERENCES

1. European Surveillance of Congenital Anomalies. Prevalence tables for all full member registries from 2011-2015. Available from: http://www.eurocat-network.eu/accessprevalencedata/prevalencetables. Published 2017. Updated 2017. Accessed 4/24, 2017.
2. Nicoletti D, Appel LD, Siedersberger Neto P, Guimaraes GW, Zhang L. Maternal smoking during pregnancy and birth defects in children: A systematic review with meta-analysis. Cad Saude Publica. 2014;30(12):2491-2529.
3. Stothard KJ, Tennant PW, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: A systematic review and meta-analysis. JAMA. 2009;301(6):636-650.
4. Vrijheid M, Martinez D, Manzanares S, et al. Ambient air pollution and risk of congenital anomalies: A systematic review and meta-analysis. Environ Health Perspect. 2011;119(5):598-606.
5. Burdorf A, Figà-Talamanca I, Jensen TK, Thulstrup AM. Effects of occupational exposure on the reproductive system: Core evidence and practical implications. Occup Med (Lond). 2006;56(8):516-520.
6. Figa-Talamanca I. Occupational risk factors and reproductive health of women. Occup Med (Lond). 2006;56(8):521-531.
7. Kumar S. Occupational exposure associated with reproductive dysfunction. J Occup Health. 2004;46(1):1-19.
8. Snijder CA, te Velde E, Roeleveld N, Burdorf A. Occupational exposure to chemical substances and time to pregnancy: A systematic review. Hum Reprod Update. 2012;18(3):284-300.
9. Fritschi L, Siemiatycki J, Richardson L. Self-assessed versus expert-assessed occupational exposures. Am J Epidemiol. 1996;144(5):521-527.
10. Nieuwenhuijsen MJ. Exposure assessment in occupational and environmental epidemiology. Online edition ed. Oxford, United Kingdom: Oxford University Press; 2003. Available from: http://www.oxfordscholarship.com/view/10.1093/acprof:oso/9780198528616.001.0001/acprof-9780198528616.
11. Pannett B, Coggon D, Acheson ED. A job-exposure matrix for use in population based studies in england and wales. Br J Ind Med. 1985;42(11):777-783.
12. Higgins JPT GS, ed. Cochrane handbook for systematic reviews of interventions Version 5.1.0 [Updated March 2011]. Available from: www.cochrane-handbook.org: The Cochrane Collaboration; 2011.
13. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. PLoS Med. 2009;6(7):e1000100.
14. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 4/21, 2017.
15. Kuiper JS, Zuidersma M, Oude Voshaar RC, et al. Social relationships and risk of dementia: A systematic review and meta-analysis of longitudinal cohort studies. Ageing Res Rev. 2015;22:39-57.
16. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629-634.

17. Garlantézec R, Monfort C, Rouget F, Cordier S. Maternal occupational exposure to solvents and congenital malformations: A prospective study in the general population. Occup Environ Med. 2009;66(7):456-463.

18. Morales-Suarez-Varela MM, Toft GV, Jensen MS, et al. Parental occupational exposure to endocrine disrupting chemicals and male genital malformations: A study in the Danish national birth cohort study. Environ Health. 2011;10(1):3-3.

19. Blatter BM, Roeleveld N, Zielhuis GA, Gabreels FJ, Verbeek AL. Maternal occupational exposure during pregnancy and the risk of spina bifida. Occup Environ Med. 1996;53(2):80-86.

20. Brender J, Suarez L, Hendricks K, Baetz RA, Larsen R. Parental occupation and neural tube defect-affected pregnancies among Mexican Americans. J Occup Environ Med. 2002;44(7):650-656.

21. Cordier S, Szabova E, Fevotte J, Bergeret A, Plackova S, Mandereau L. Congenital malformations and maternal exposure to glycol ethers in the Slovak republic. Epidemiology. 2001;12(5):592-593.

22. Cordier S, Bergeret A, Goujard J, et al. Congenital malformation and maternal occupational exposure to glycol ethers. occupational exposure and congenital malformations working group. Epidemiology. 1997;8(4):355-363.

23. Desrosiers TA, Lawson CC, Meyer RE, et al. Maternal occupational exposure to organic solvents during early pregnancy and risks of neural tube defects and orofacial clefts. Occup Environ Med. 2012;69(7):493-499.

24. Makelarski JA, Romitti PA, Rocheleau CM, et al. Maternal periconceptional occupational pesticide exposure and neural tube defects. Birth Defects Res Part A Clin Mol Teratol. 2014;100(11):877-886.

25. Pettigrew SM, Bell EM, Van Zutphen AR, et al. Paternal and joint parental occupational pesticide exposure and spina bifida in the national birth defects prevention study, 1997 to 2002. Birth Defects Res Part A Clin Mol Teratol. 2016;106(11):963-971.

26. Shaw GM, Wasserman CR, O'Malley CD, Nelson V, Jackson RJ. Maternal pesticide exposure from multiple sources and selected congenital anomalies. Epidemiology. 1999;10(1):60-66.

27. Brender JD, Suarez L, Felkner M, et al. Maternal exposure to arsenic, cadmium, lead, and mercury and neural tube defects in offspring. Environ Res. 2006;101(1):132-139.

28. Cordier S, Ha MC, Ayme S, Goujard J. Maternal occupational exposure and congenital malformations. Scand J Work Environ Health. 1992;18(1):11-17.

29. Gilboa SM, Desrosiers TA, Lawson C, et al. Association between maternal occupational exposure to organic solvents and congenital heart defects, national birth defects prevention study, 1997-2002. Occup Environ Med. 2012;69(9):628-635.

30. Tikkanen J, Kurppa K, Timonen H, Holmberg PC, Kuosma E, Rantala K. Cardiovascular malformations, work attendance, and occupational exposures during pregnancy in Finland. Am J Ind Med. 1988;14(2):197-204.

31. Rocheleau CM, Bertke SJ, Lawson CC, et al. Maternal occupational pesticide exposure and risk of congenital heart defects in the national birth defects prevention study. Birth Defects Res Part A Clin Mol Teratol. 2015;103(10):823-833.

32. Snijder CA, Vlot IJ, Burdorf A, et al. Congenital heart defects and parental occupational exposure to chemicals. Hum Reprod. 2012;27(5):1510-1517.

33. Wang C, Zhan Y, Wang F, et al. Parental occupational exposures to endocrine disruptors and the risk of simple isolated congenital heart defects. Pediatr Cardiol. 2015;36(5):1024-1037.

34. Jackson LW, Correa-Villaseñor A, Lees PSJ, et al. Parental lead exposure and total anomalous pulmonary venous return. Birth Defects Res Part A Clin Mol Teratol. 2004;70(4):185-193.

35. Chevrier C, Dananché B, Bahuau M, et al. Occupational exposure to organic solvent mixtures during pregnancy and the risk of non-syndromic oral clefts. Occup Environ Med. 2006;63(9):617-623.

36. Lorente C, Cordier S, Bergeret A, et al. Maternal occupational risk factors for oral clefts. Scand J Work Environ Health. 2000;26(2):137-145.

37. Spinder N, Bergman JEH, Boezen HM, Vermeulen RCH, Kromhout H, de Walle, H E K. Maternal occupational exposure and oral clefts in offspring. Environ Health. 2017;16(1):83-5.

38. Kalfa N, Paris F, Philibert P, et al. Is hypospadias associated with prenatal exposure to endocrine disruptors? A French collaborative controlled study of a cohort of 300 consecutive children without genetic defect. Eur Urol. 2015;68(6):1023-1030.

39. Carbone P, Giordano F, Nori F, et al. The possible role of endocrine disrupting chemicals in the aetiology of cryptorchidism and hypospadias: A population-based case-control study in rural Sicily. Int J Androl. 2007;30(1):3-13.

40. Giordano F, Abballe A, De Felip E, et al. Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. Birth Defects Res Part A Clin Mol Teratol. 2010;88(4):241-250.

41. Nassar N, Abeywardana P, Barker A, Bower C. Parental occupational exposure to potential endocrine disrupting chemicals and risk of hypospadias in infants. Occup Environ Med. 2010;67(9):585-589.

42. Pierik FH, Burdorf A, Deddens JA, Juttmann RE, Weber RF. Maternal and paternal risk factors for cryptorchidism and hypospadias: A case-control study in newborn boys. Environ Health Perspect. 2004;112(15):1570-1576.

43. Rocheleau CM, Romitti PA, Sanderson WT, et al. Maternal occupational pesticide exposure and risk of hypospadias in the national birth defects prevention study. Birth Defects Res Part A Clin Mol Teratol. 2011;91(11):927-936.

44. Vrijheid M, Armstrong B, Dolk H, Van Tongeren M, Botting B. Risk of hypospadias in relation to maternal occupational exposure to potential endocrine disrupting chemicals. Occup Environ Med. 2003;60(8):543-550.

45. European Surveillance of Congenital Anomalies. EUROCAT guide 1.4 and reference documents. Available from: http://www.eurocat-network.eu/content/EUROCAT-Guide-1.4-Full-Guide.pdf. Published 2013. Updated 2013. Accessed 9/9, 2017.

46. Hansen M, Kurinczuk JJ, Milne E, de Klerk N, Bower C. Assisted reproductive technology and birth defects: A systematic review and meta-analysis. Hum Reprod Update. 2013;19(4):330-353.

47. Shi L, Chia SE. A review of studies on maternal occupational exposures and birth defects, and the limitations associated with these studies. Occup Med (Lond). 2001;51(4):230-244.

48. Kromhout H, Vermeulen R. Application of job-exposure matrices in studies of the general population: Some clues to their performance. Eur Respir Rev. 2001(11):80-90.

49. 't Mannetje A, Kromhout H. The use of occupation and industry classifications in general population studies. Int J Epidemiol. 2003;32(3):419-428.

50. Blair A, Stewart P, Lubin JH, Forastiere F. Methodological issues regarding confounding and exposure misclassification in epidemiological studies of occupational exposures. Am J Ind Med. 2007;50(3):199-207.

51. Ioannidis JP, Trikalinos TA. The appropriateness of asymmetry tests for publication bias in meta-analyses: A large survey. CMAJ. 2007;176(8):1091-1096.

52. Rocheleau CM, Romitti PA, Dennis LK. Pesticides and hypospadias: a meta-analysis. J Pediatr Urol. 2009;5(1):17-24.

53. Romitti PA, Herring AM, Dennis LK, Wong-Gibbons D. Meta-analysis: Pesticides and orofacial clefts. Cleft Palate-Craniofac J. 2007;44(4):358-365

SUPPLEMENTARY FILES

Supplementary File 1

Search strategy: PUBMED

("Environmental Exposure"[Mesh] OR "Toxic Actions"[Mesh] OR "Radiation"[Mesh] OR "Prenatal Exposure Delayed Effects"[Mesh] OR exposure*[tiab] OR exposed [tiab] OR hazard*[tiab] OR radiation [tiab] OR "toxicity" [Subheading] OR "poisoning" [Subheading] OR teratogen* [tiab])

AND

("Work"[Mesh] OR "Workplace"[Mesh] OR "Occupational Exposure"[Mesh] OR "Air Pollutants, Occupational"[Mesh] OR occupation*[tw] OR work*[tiab] OR employee*[tiab] OR personnel[tiab])

AND

("Congenital Abnormalities"[Mesh] OR abnormalit*[tiab] OR birth defect*[tiab] OR deformit*[tiab] OR neural tube[tiab] OR cleft palate[tiab] OR cleft lip[tiab] OR oral cleft*[tiab] OR hypospad*[tiab] OR anomal*[tiab] OR congenital [tiab] OR malformat* [tiab])

AND

("Maternal Exposure"[Mesh] OR "Pregnancy" [Mesh] OR "Embryonic and Fetal Development" [Mesh] OR offspring[tiab] OR maternal[tiab] OR mother*[tiab] OR parent*[tiab] OR conception*[tiab] OR periconception*[tiab] OR birth[tiab] OR pregnan* [tiab] OR prenatal*[tiab] OR fetal[tiab] OR foetal[tiab] OR reproduct* [tiab] OR outcome [tiab])

NOT

(("Animals"[Mesh] NOT "Humans"[Mesh]) OR animal[ti] OR mice[ti] OR mouse[ti] OR rat[ti] OR rats[ti])

Search strategy: EMBASE

('exposure'/exp OR 'environmental, industrial and domestic chemicals'/exp OR 'teratogenesis'/exp OR 'electromagnetic field'/exp OR 'electric field'/exp OR 'radiation'/exp OR 'radiation and radiation related phenomena'/exp OR 'radiation related phenomena'/exp OR 'polycyclic aromatic hydrocarbon'/exp OR 'endocrine disruptor'/exp OR (exposure* OR exposed OR hazard* OR radiation OR toxicity OR toxic OR poisoning OR teratogen*):ab,ti)

AND

('occupation and occupation related phenomena'/exp OR 'occupational disease'/exp OR (occupation* OR 'at work' OR worker* OR 'work related' OR 'work environment' OR 'mothers work*' OR 'maternal work*' OR workplace OR 'work place' OR employee* OR personnel):ab,ti OR (mother* NEXT/2 work*) OR (women* NEXT/2 work*):ab,ti)

AND

('congenital disorder'/exp OR (abnormalit* OR anomal* OR 'birth defect*' OR deformit* OR 'neural tube' OR 'cleft palate' OR 'cleft lip' OR 'oral cleft*' OR hypospad* OR congenital OR malformat*):ab,ti)

AND

('maternal exposure'/exp OR 'pregnancy'/exp OR 'prenatal development'/exp OR 'prenatal drug exposure'/exp OR 'prenatal exposure'/exp OR 'prenatal period'/exp OR (offspring OR maternal OR mother* OR parent* OR conception* OR periconception* OR birth OR pregnan* OR prenatal* OR fetal OR foetal OR reproduct* OR outcome):ab,ti)

NOT

((('animal'/exp OR 'nonhuman'/exp) NOT 'human'/exp) OR (animal OR mice OR mouse OR rat OR rats):ti)

Supplementary File 2

Newcastle-Ottawa Quality Assessment Scale for Case-control studies

- 'High' quality choices were identified with a star.
- Maximum of 9 stars (low risk of bias), minimum of 0 stars (high risk of bias).
- A maximum of one star for each item within the *Selection* and *Exposure/Outcome* categories. A maximum of two stars for *Comparability*

Scales		Stars	Manual
Selection (max. 4 stars)			
1	Is the case definition adequate? a) yes, with independent validation b) yes, e.g. record linkage or based on self-reports a) c) no description	* - - 	a) Requires some independent validation (e.g. >1 person/record/time/process to extract information, or reference to primary record source such as x-rays or medical/hospital records) b) Record linkage (e.g. ICD codes in database) or self-report with no reference to primary record c) No description
2	Representativeness of the cases a) consecutive or obviously representative series of cases b) potential for selection biases or not stated	* -	a) All eligible cases with outcome of interest over a defined period of time, all cases in a defined catchment area, all cases in a defined hospital or clinic, group of hospitals, health maintenance organization, or an appropriate sample of those cases (e.g. random sample) *Surveillance systems, like birth defects registries were assumed to be low risk of bias* b) Not satisfying requirements in part (a), or not stated.
3	Selection of Controls a) community controls b) hospital controls or malformed controls c) no description	* * - 	a) Community controls (i.e. same community as cases and would be cases if had outcome) b) Hospital controls, within same community as cases (i.e. not another city) but derived from a hospitalized population. *Hospital controls were assumed to be low risk of bias, because often the next live birth is selected as control, which is commonly born in a hospital. Furthermore, malformed controls are assumed to be low risk of bias, because of low chance of recall bias.* c) No description
4	Definition of Controls a) no history of disease (endpoint) b) no description of source	* -	a) If cases are first occurrence of outcome, then it must explicitly state that controls have no history of this outcome. If cases have new (not necessarily first) occurrence of outcome, then controls with previous occurrences of outcome of interest should not be excluded. b) No mention of history of outcome *Malformed controls were considered as high risk of bias, because they have a history of disease.*

Scales		Stars	Manual
Comparability (max. 2 stars)			
1	Comparability of cases and controls on the basis of the design or analysis	*	Either cases and controls must be matched in the design and/or confounders must be adjusted for in the analysis. Statements of no differences between groups or that differences were not statistically significant are not sufficient for establishing comparability. *Maternal education, smoking or alcohol use during pregnancy, maternal body mass index, social economic status, or parity.*
	a) study controls for *maternal age, registry site (if applicable), folic acid use (in case of neural tube defects) and child sex (in case of oral clefts)*	*	
	b) study controls for any additional factor		
Exposure (max 3 stars)			
1	Ascertainment of exposure	*	Allocation of stars as per rating sheet. *Using a job exposure matrix for exposure assessment is assumed to be low risk of bias when job coding is blinded to case-control status.*
	a) secure record (e.g. medical records)		
	b) structured interview where blind to case/control status	*	
	c) interview not blinded to case/control status	-	
	d) written self-report only	-	
	e) no description	-	
2	Same method of ascertainment for cases and controls	*	
	a) yes	-	
	b) no		
3	Non-Response rate	*	Allocation of stars as per rating sheet. *A rate difference for inclusion of cases and controls is defined of a non-response rate difference of >10%.*
	a) same rate for both groups (cases/controls)	-	
	b) non respondents described		
	c) rate different and no designation	-	

Supplementary File 3

Newcastle-Ottawa Quality Assessment Scale for Cohort studies

- 'High' quality choices were identified with a star.
- Maximum 9 stars (low risk of bias), minimum 0 stars (high risk of bias).
- A maximum of one star for each item within the *Selection* and *Exposure/Outcome* categories. A maximum of two stars for *Comparability*

Scales		Stars	Manual
Selection (max. 4 stars)			
1	Representativeness of the exposed cohort a) truly representative of the average in the community b) somewhat representative of the average in the community c) selected group of users e.g. nurses, volunteers d) no description of the derivation of the cohort	* * - - -	Item is assessing the representativeness of exposed individuals in the community, not the representativeness of the sample of women from some general population. For example, subjects derived from groups likely to contain middle class, better educated, health oriented women are likely to be representative of postmenopausal estrogen users while they are not representative of all women (e.g. members of a health maintenance organization (HMO) will be a representative sample of estrogen users. While the HMO may have an under-representation of ethnic groups, the poor, and poorly educated, these excluded groups are not the predominant users of estrogen).
2	Selection of the non exposed cohort a) drawn from the same community as the exposed cohort b) drawn from a different source c) no description of the derivation of the non exposed cohort	* - - -	Allocation of stars as per rating sheet
3	Ascertainment of exposure a) secure record (e.g. medical records) b) structured interview c) written self-report d) no description	* * - -	Allocation of stars as per rating sheet
4	Demonstration that outcome of interest was not present at start of study a) yes b) no	* -	In the case of mortality studies, outcome of interest is still the presence of a disease/ incident, rather than death. That is to say that a statement of no history of disease or incident earns a star.
Comparability (max. 2 stars)			

Scales		Stars	Manual
1	Comparability of cohorts on the basis of the design or analysis	*	Either cases and controls must be matched in the design and/or confounders must be adjusted for in the analysis. Statements of no differences between groups or that differences were not statistically significant are not sufficient for establishing comparability. *Maternal education, smoking or alcohol use during pregnancy, maternal body mass index, social economic status, or parity.*
	a) study controls for *maternal age, registry site (if applicable), folic acid use (in case of neural tube defects) and child sex (in case of oral clefts)*	*	
	b) study controls for any additional factor		
Exposure (max 3 stars)			
1	Ascertainment of exposure	*	Allocation of stars as per rating sheet *Using a job exposure matrix for exposure assessment is assumed to be low risk of bias when job coding is blinded to case-control status.*
	a) independent blind assessment	*	
	b) record linkage	-	
	c) self-report	-	
	d) no description		
2	Was follow-up long enough for outcomes to occur	*	*Follow up time of one year*
	a) yes	-	
	b) no		
3	Adequacy of follow up of cohorts	*	Allocation of stars as per rating sheet
	a) complete follow up - all subjects accounted for	*	
	b) subjects lost to follow up unlikely to introduce bias - small number lost - <10 %	-	
	c) lost to follow up rate >10% and no description of those lost	-	
	d) no statement		

Supplementary Table 1 | Study characteristics and results of the 28 studies included in the systematic review

Study	Type of congenital anomalies	Exclusion criteria for cases with congenital anomalies	Exposure	Congenital anomalies		Controls		OR (original data)	95% CI (original data)	OR (calculated crude ORs)	95% CI (calculated crude ORs)
				Exposed	Total	Exposed	Total				
Blatter et al. (1996)	Spina bifida aperta	-	Organic solvents	29	274	35	314	0.90	0.60-1.60	1.15	0.16-8.20
			Pesticides	2	274	2	314	NA	NA		
			Mercury	2	274	11	314	0.20	0.10-0.80		
Brender et al. (2002)	NTD	-	Solvents*	9	184	0	225	∞	2.4-∞		
			Glycol ethers*	7	184	0	225	∞	1.8-∞		
			Pesticides	4	184	4	225	1.20	0.30-4.80		
			Lead*	3	184	4	225	1.1	0.2-5.8		
Brender et al. (2006)	NTD	-	Heavy metals	10	184	7	225	NA	NA	1.79	0.67-4.80
Carbone et al. (2006)	Hypospadias		Pesticides	2	43	16	203	NA	NA	0.57	0.13-2.58
Chevrier et al. (2006)	Non-syndromic oral clefts	Syndromic cases including Pierre Robin sequence	Organic solvents	90	173	73	187	NA	NA	1.69	1.11-2.57
			Glycol ethers	55	138	41	155	NA	NA	1.84	1.12-3.02
Cordier et al. (1992)	CHD	Malformations of known environmental or familial origin	Solvents	6	22	4	21	1.30	0.30-6.20[a]		
	Oral clefts			8	14	3	19	6.80	0.70-128.30[a]		

Supplementary Table 1. Continued

Study	Type of congenital anomalies	Exclusion criteria for cases with congenital anomalies	Exposure	Congenital anomalies		Controls		OR (original data)	95% CI (original data)	OR (calculated crude ORs)	95% CI (calculated crude ORs)
				Exposed	Total	Exposed	Total				
Cordier et al. (1997)	NTD	All genetic syndromes (Mendelian and chromosomal)	Glycol ethers	28	94	137	751	1.94	1.16-3.24		
	CHD			56	249	137	751	1.45	0.99-2.13		
	Oral clefts			34	100	137	751	1.97	1.20-3.25		
Cordier et al. (2001)	NTD	-	Glycol ethers	1	16	5	131	NA	NA	1.68	0.18-15.36
	CHD			5	42	5	131	3.20	0.80-12.00		
	Oral clefts			2	18	5	131	3.90	0.60-25.00		
Desrosiers et al. (2012)	NTD	Pre-gestational diabetes, family history of NTDs or OFCs, known etiology (single-gene disorders and chromosomal anomalies)	Organic solvents	66	504	242	2949	NA	NA	1.69	1.26-2.25
	Oral clefts			111	1154	242	2946	NA	NA	1.19	0.94-1.51
Garlantézec et al. (2009)	CHD	Genetic and chromosomal anomalies	Solvents	1	6	576	2730	0.75	0.10-6.50		
	Oral clefts			6	8	576	2730	12.85	2.60-64.70		

Supplementary Table 1. Continued

Study	Type of congenital anomalies	Exclusion criteria for cases with congenital anomalies	Exposure	Congenital anomalies		Controls		OR (original data)	95% CI (original data)	OR (calculated crude ORs)	95% CI (calculated crude ORs)
				Exposed	Total	Exposed	Total				
Gilboa et al. (2012)	CHD (isolated)	Single gene conditions, chromosome abnormalities First degree family history of CHD, mothers with pregestational diabetes, mothers of cases with extracardiac defects or cases with associated or complex CHDs	Organic solvents	96	2047	110	2951	1.20	0.90-1.60		
Giordano et al. (2010)	Hypospadias	-	Pesticides*	4	80	0	80	NA	NA	NA	NA
			Heavy metals	5	80	3	80	1.99	0.44-8.97		
Jackson et al. (2004)	(Total anomalous pulmonary venous return (TAPVR) (CHD)	Twins, non-cardiac birth defects, race other than black or white, or low birth weight	Lead	4	54	41	522	0.94	0.23-2.75		
Kalfa et al. (2015)	Isolated hypospadias	Familial form with vertical transmission or mutations	Organic solvents	48	300	15	302	3.63	1.94-7.17		
			Pesticides	27	300	13	302	2.20	1.07-4.74		

Supplementary Table 1. Continued

Study	Type of congenital anomalies	Exclusion criteria for cases with congenital anomalies	Exposure	Congenital anomalies		Controls		OR (original data)	95% CI (original data)	OR (calculated crude ORs)	95% CI (calculated crude ORs)
				Exposed	Total	Exposed	Total				
Lorente et al. (2000)	Oral clefts	-	Glycol ethers*	34	100	137	751	NA	NA	NA	NA
			Lead	11	100	45	751	NA	NA	1.94	0.97-3.89
Makelarski et al. (2014)	NTD	Chromosomal or single gene disorders, maternal diagnosis of diabetes before or during pregnancy, maternal periconceptional exposure to folic acid antagonists	Pesticides	162	496	888	2930	0.90	0.70-1.10		
Morales-Suarez-Varela et al. (2011)	Hypospadias	Not intending carry pregnancy to term	Pesticides	2	244	309	45079	NA	NA	1.20	0.30-4.84
			Heavy metals	4	244	612	45079	NA	NA	1.21	0.45-8.26
Nassar et al. (2009)	Hypospadias	-	Pesticides	17	1075	37	2289	0.92	0.50-1.70		
			Heavy metals	18	1075	16	2289	2.59	1.28-5.23		
Pettigrew et al. (2016) *	Spina bifida	Family history of NTDs, maternal type 1 or type 2 diabetes mellitus, or mothers' who used medication known to be associated with birth defects	Pesticides	84	267	797	2542				

Supplementary Table 1. Continued

Study	Type of congenital anomalies	Exclusion criteria for cases with congenital anomalies	Exposure	Congenital anomalies		Controls		OR (original data)	95% CI (original data)	OR (calculated crude ORs)	95% CI (calculated crude ORs)
				Exposed	Total	Exposed	Total				
Pierik et al. (2004)	Hypospadias	-	Pesticides	2	56	7	313	1.60	0.20-6.90		
Rocheleau et al. (2011)	Hypospadias (second and third degree)	Epispadias, ambiguous genitalia (with female karyotype), or chordae alone	Pesticides	140	559	464	1443	0.78	0.61-1.01		
Rocheleau et al. (2015)	CHD	Known syndromes or suspected chromosomal abnormalities	Pesticides	1045	3318	916	2979	1.04	0.93-1.15		
Shaw et al. (1999)	NTD	Monogenic conditions, trisomy, Turner, or NTD cases with amniotic band pathogenesis	Pesticides	15	143	24	265	NA	NA	1.18	0.60-2.32
	Conotruncal heart defects			7	133	24	265	NA	NA	0.56	0.23-1.33
	Oral clefts (isolated)			25	257	43	417	NA	NA	0.94	0.56-1.58
Snijder et al. (2012)	CHD	-	Pesticides	6	424	9	480	0.25	0.05-1.36		
			Heavy metals	3	424	4	480	1.40	0.29-6.74		
Spinder et al. (2017)	Oral clefts	Chromosomal disorders, monogenic disorders, anencephaly, arhinencephaly and holoprosencephaly	Solvents	103	387	1075	4356	1.10	0.90-1.40		
			Pesticides	14	387	88	4356	1.70	1.00-3.10		
			Heavy metals	4	387	44	4356	1.10	0.40-3.00		

Supplementary Table 1. Continued

Study	Type of congenital anomalies	Exclusion criteria for cases with congenital anomalies	Exposure	Congenital anomalies		Controls		OR (original data)	95% CI (original data)	OR (calculated crude ORs)	95% CI (calculated crude ORs)
				Exposed	Total	Exposed	Total				
Tikkanen et al. (1988)	CHD	Chromosomal anomalies and uncertain diagnosis	Organic solvents	21	160	16	160	NA	NA	1.36	0.68-2.71
			Pesticides	1	160	4	160			0.25	0.03-2.22
Vrijheid et al. (2002)	Hypospadias	Chromosomal abnormalities	Pesticides	37	3471	344	32491	NA	NA	1.01	0.72-1.42
			Heavy metals	62	3471	672	32491	NA	NA	0.86	0.66-1.12
Wang et al. (2015)	CHD (isolated)	Complex CHD, combined CHD, family history, pre-gestational diabetes	Pesticides	29	707	17	593	0.90	0.50-1.80		
			Heavy metals	20	707	3	593	4.90	1.40-17.10		

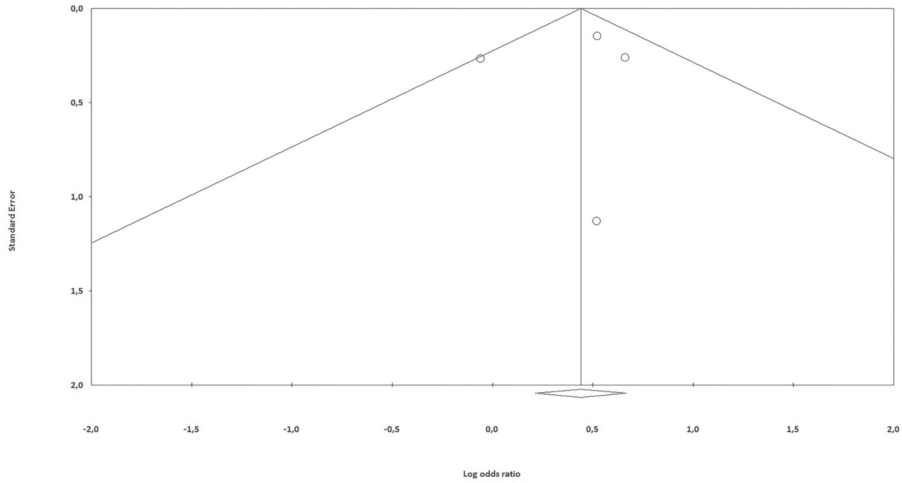
NTD = Neural Tube Defect, CHD = Congenital Heart Defect, * only considered for meta-analysis, [a] = 90% CI.

Supplementary Table 2 | Quality assessment of included studies using the Newcastle-Ottawa quality assessment [a]

Study	Study design	Selection				Comparability	Exposure			Total
		S1	S2	S3	S4	C	E1	E2	E3	
Blatter et al. (1996)	Case-control	*	*	*	*	**	-	*	*	8
Brender et al. (2002)	Case-control	*	*	*	*	**	*	*	-	8
Brender et al. (2006)	Case-control	*	*	*	*	--	*	*	-	6
Carbone et al. (2006)	Case-control	*	*	*	*	**	*	*	-	8
Chevrier et al. (2006)	Case-control	*	*	*	*	**	*	*	*	9
Cordier et al. (1992)	Case-control	*	*	*	*	**	*	*	-	8
Cordier et al. (1997)	Case-control	*	*	*	*	**	*	*	-	8
Cordier et al. (2001)	Case-control	-	-	-	*	*	*	*	-	4
Desrosiers et al. (2012)	Case-control	*	*	*	*	**	*	*	*	9
Garlantézec et al. (2009)	Cohort	-	*	*	*	**	*	*	*	9
Gilboa et al. (2012)	Case-control	*	*	*	*	*	*	*	-	8
Giordano et al. (2010)	Case-control	*	*	*	*	*	*	*	-	8
Jackson et al. (2004)	Case-control	*	-	*	*	--	*	*	-	5
Kalfa et al. (2015)	Case-control	*	-	*	*	--	-	*	-	4
Lorente et al. (2000)	Case-control	*	*	*	*	*	*	*	*	8
Makelarski et al. (2014)	Case-control	-	-	*	*	*	-	*	*	5
Morales-Suarez-Varela et al. (2011)	Cohort	*	*	*	*	**	*	*	-	8
Nassar et al. (2009)	Case-control	*	*	*	*	**	*	*	*	9
Pettigrew et al. (2016)	Case-control	*	-	*	*	--	*	*	-	5
Pierik et al. (2004)	Case-control	*	*	*	*	--	*	*	-	6
Rocheleau et al. (2011)	Case-control	*	*	*	*	--	-	*	*	6
Rocheleau et al. (2015)	Case-control	*	*	*	*	**	-	*	*	8
Shaw et al. (1999)	Case-control	*	-	*	*	--	*	*	*	6
Snijder et al. (2012)	Case-control	*	*	*	*	**	*	*	-	8
Spinder et al. (2017)	Case-control	*	*	*	-	*	*	*	-	6
Tikkanen et al. (1988)	Case-control	*	-	*	-	--	*	*	-	4
Vrijheid et al. (2002)	Case-control	*	*	*	-	**	*	*	-	7
Wang et al. (2015)	Case-control	*	*	*	*	**	-	*	-	7

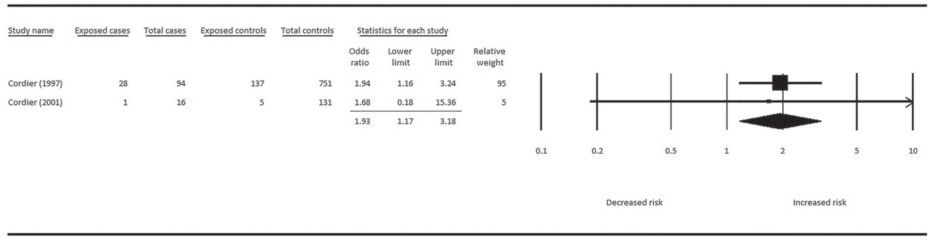
[a] A maximum of nine stars can be allocated to each study. If the maximum number of stars was assigned, the study was considered as having low risk of bias. S1: adequate case definition/representativeness of the exposed cohort. S2: representativeness of the cases/selection of the non-affected cohort drawn from the same community as affected cohort. S3: selection of controls from community/adequate ascertainment of exposure. S4: definition of controls/demonstration that outcome of interest was not present at start of study. C: comparability of cases and controls on the basis of the design or analysis/comparability of cohorts on the basis of the design or analysis. E1: ascertainment of exposure. E2: same method of ascertainment for cases and controls/ follow-up long enough for outcomes to occur. E3: non-response rate/adequacy of follow up of cohorts.

Supplementary Figure 1 | Funnel plot of maternal occupational exposure to solvents and risk of neural tube defects in offspring



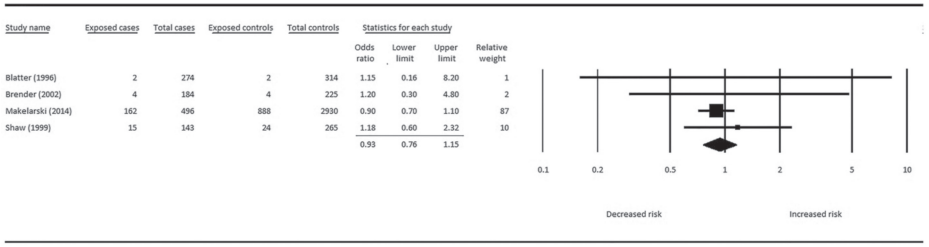
p-value = 0.40

Supplementary Figure 2 | Forest plot of maternal occupational exposure to glycol ethers and risk of neural tube defects in offspring



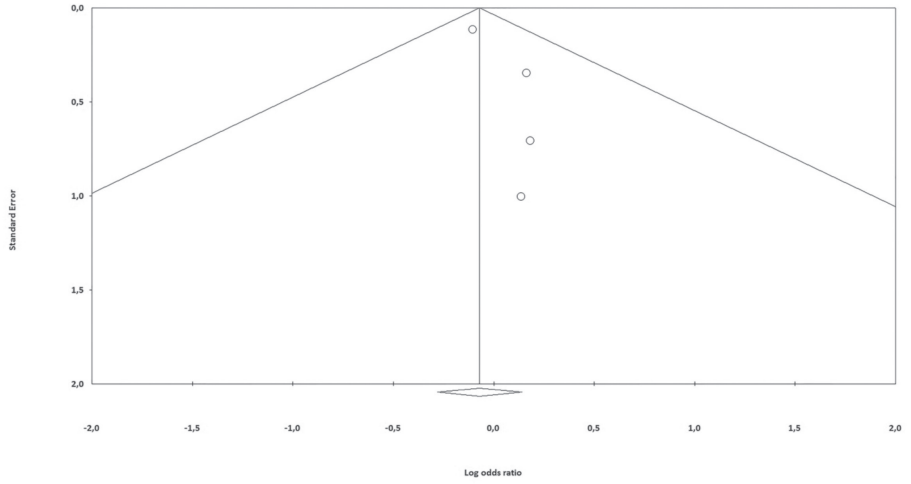
Heterogeneity: $\chi^2 = 1.54$, df = 1, $P = 0.90$, $I^2 = 0\%$

Supplementary Figure 3 | Forest plot of maternal occupational exposure to pesticides and risk of neural tube defects in offspring



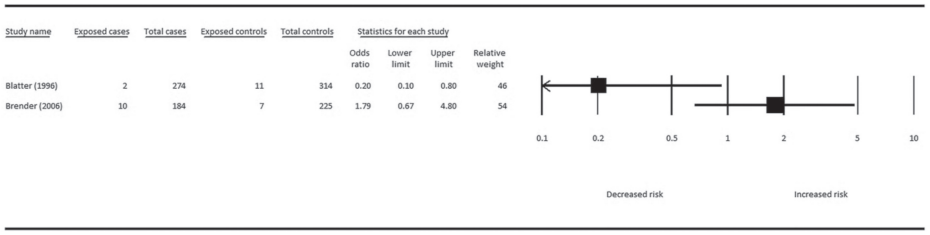
Heterogeneity: $\chi^2 = 0.71$, df = 3, $P = 0.87$, $I^2 = 0\%$

Supplementary Figure 4 | Funnel plot of maternal occupational exposure to pesticides and risk of neural tube defects in offspring



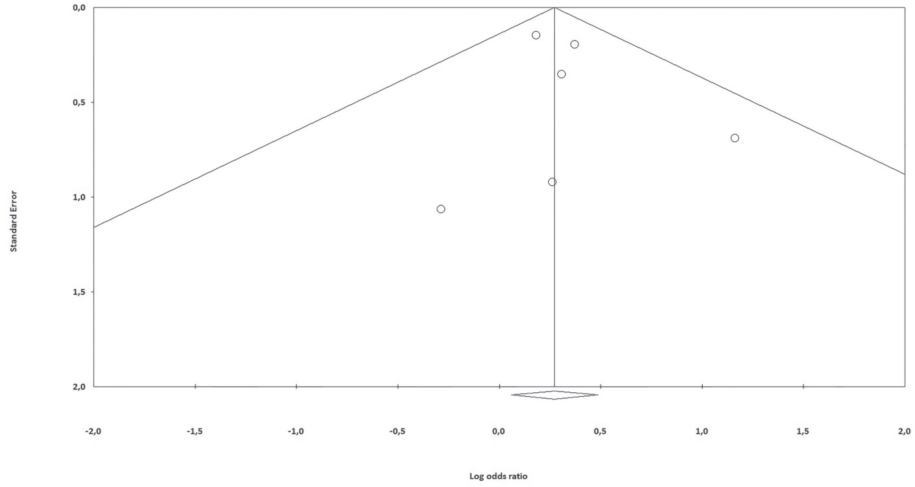
p-value = 0.08

Supplementary Figure 5 | Forest plot of maternal occupational exposure to metals and risk of neural tube defects in offspring



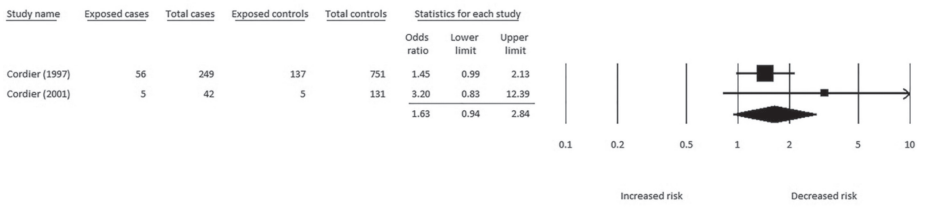
Heterogeneity: $\chi^2 = 5.58$, df = 1, $P = 0.02$, $I^2 = 82\%$

Supplementary Figure 6 | Funnel plot of maternal occupational exposure to solvents and risk of congenital heart defects in offspring



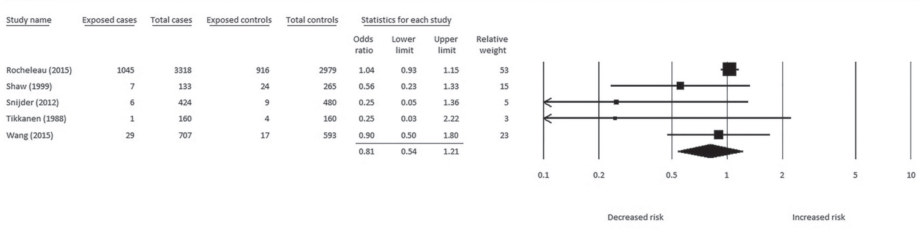
p-value = 0.27

Supplementary Figure 7 | Forest plot of maternal occupational expsoure to glycol ethers and risk of congenital heart defects in offspring



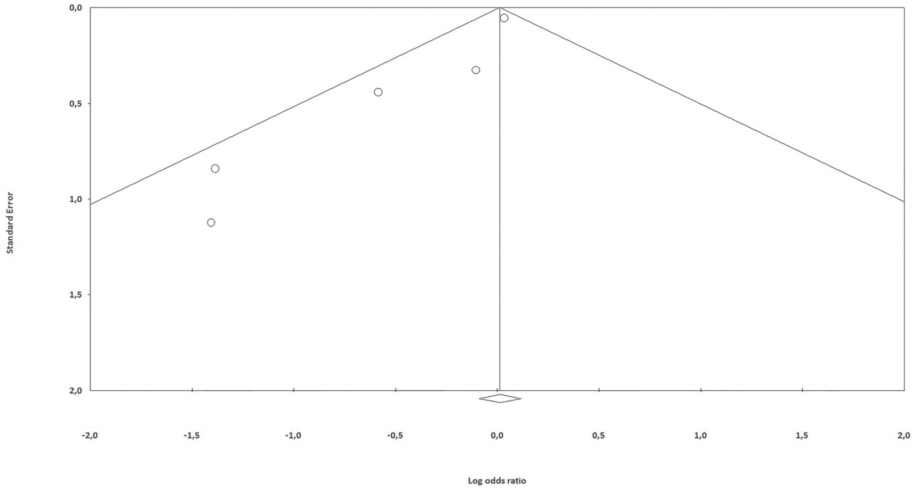
Heterogeneity: $\chi^2 = 1.22$, df = 1, $P = 0.27$, $I^2 = 18\%$

Supplementary Figure 8 | Forest plot of maternal occupational exposure to pesticides and risk of congenital heart defects in offspring



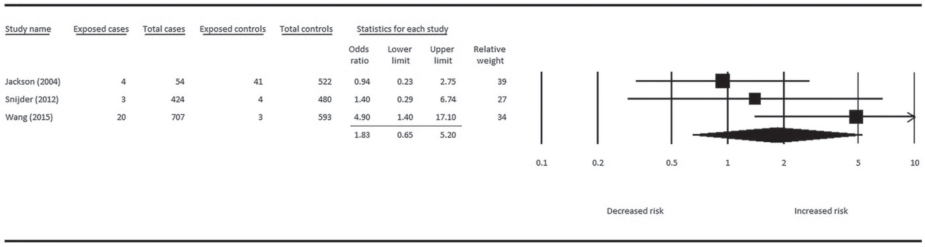
Heterogeneity: $\chi^2 = 6.45$, df = 4, $P = 0.17$, $I^2 = 38\%$

Supplementary Figure 9 | Funnel plot of maternal occupational exposure to pesticides and risk of congenital heart defects in offspring



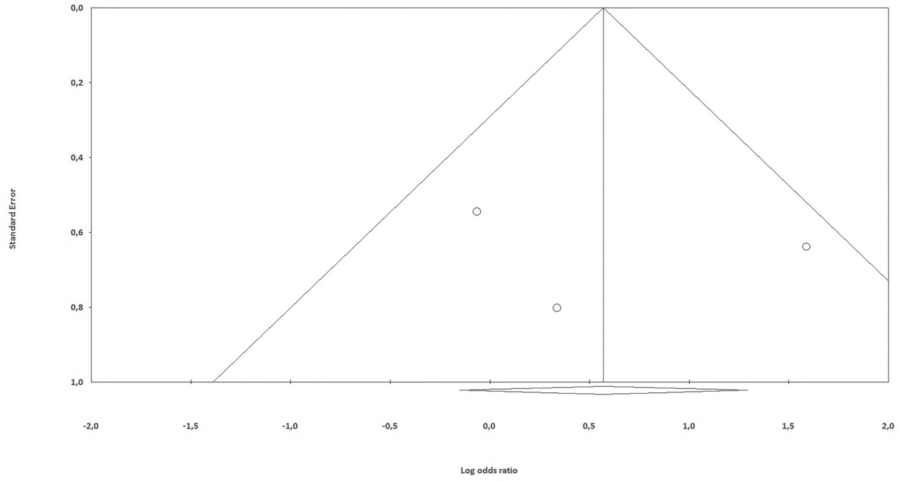
p-value = <0.01

Supplementary Figure 10 | Forest plot of maternal occupational exposure to metals and risk of congenital heart defects in offspring



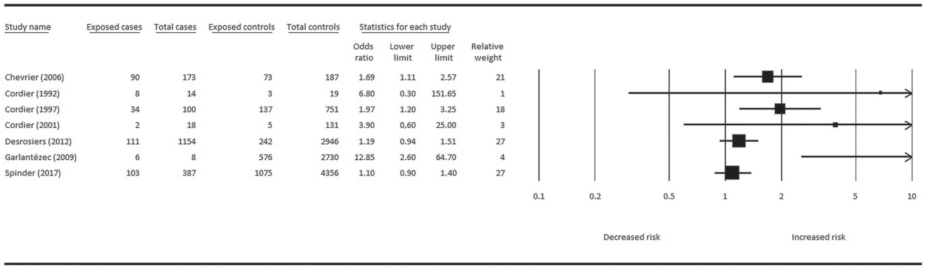
Heterogeneity: $\chi^2 = 3.99$, df = 2, $P = 0.14$, $I^2 = 49.8\%$

Supplementary Figure 11 | Funnel plot of maternal occupational exposure to metals and risk of congenital heart defects in offspring



p-value = 0.41

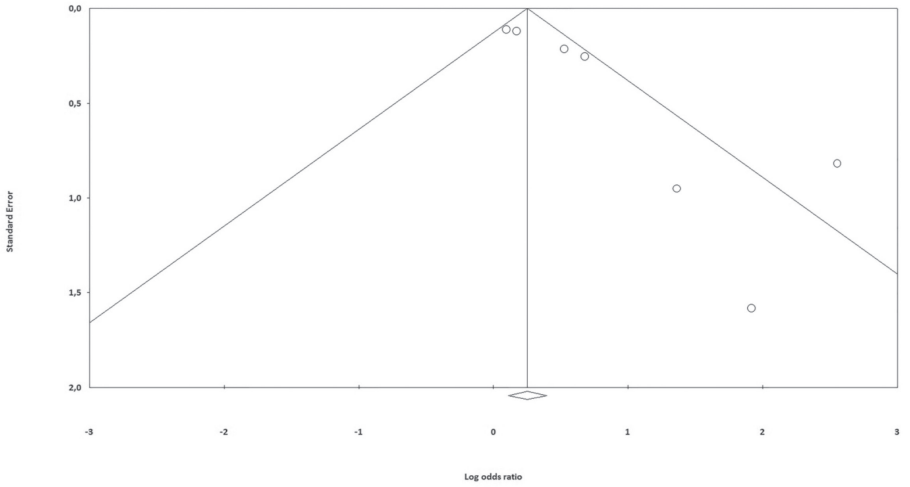
Supplementary Figure 12 | Forest plot of maternal occupational exposure to solvents and risk of oral clefts in offspring



Study name	Exposed cases	Total cases	Exposed controls	Total controls	Statistics for each study			
					Odds ratio	Lower limit	Upper limit	Relative weight
Chevrier (2006)	90	173	73	187	1.69	1.11	2.57	21
Cordier (1992)	8	14	3	19	6.80	0.30	151.65	1
Cordier (1997)	34	100	137	751	1.97	1.20	3.25	18
Cordier (2001)	2	18	5	131	3.90	0,60	25.00	3
Desrosiers (2012)	111	1154	242	2946	1.19	0.94	1.51	27
Garlantézec (2009)	6	8	576	2730	12.85	2.60	64.70	4
Spinder (2017)	103	387	1075	4356	1.10	0.90	1.40	27

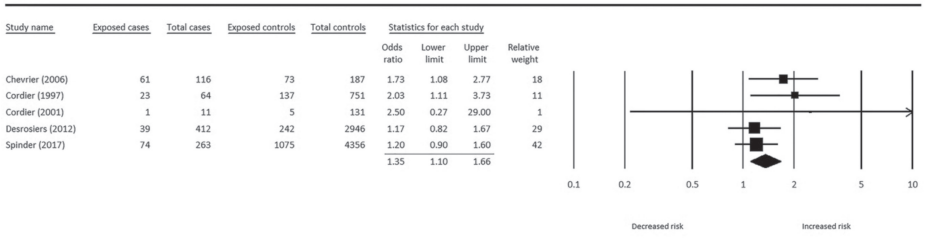
Heterogeneity: $\chi^2 = 17.17$, df = 6, $P = 0.01$, $I^2 = 65\%$

Supplementary Figure 13 | Funnel plot of maternal occupational exposure to solvents and risk of oral clefts in offspring



p-value = <0.01

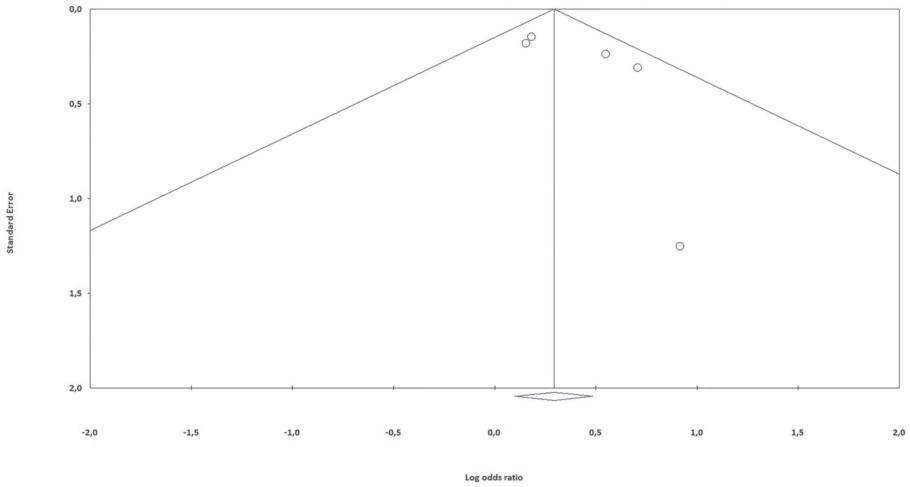
Supplementary Figure 14 | Forest plot of maternal occupational exposure to solvents and cleft lip with or without cleft palate in offspring



Study name	Exposed cases	Total cases	Exposed controls	Total controls	Statistics for each study			
					Odds ratio	Lower limit	Upper limit	Relative weight
Chevrier (2006)	61	116	73	187	1.73	1.08	2.77	18
Cordier (1997)	23	64	137	751	2.03	1.11	3.73	11
Cordier (2001)	1	11	5	131	2.50	0.27	29.00	1
Desrosiers (2012)	39	412	242	2946	1.17	0.82	1.67	29
Spinder (2017)	74	263	1075	4356	1.20	0.90	1.60	42
					1.35	1.10	1.66	

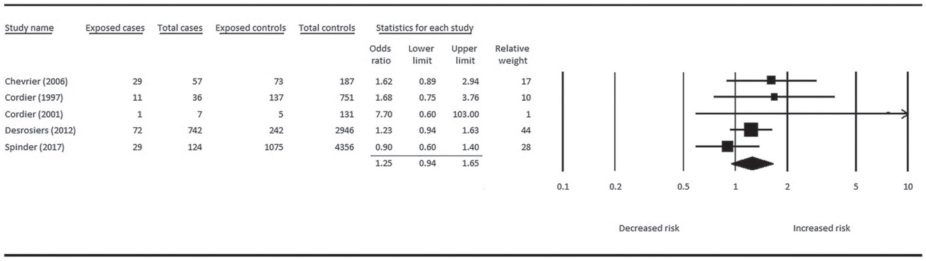
Heterogeneity: $\chi^2 = 4.35$, df = 4, $P = 0.36$, $I^2 = 8\%$

Supplementary Figure 15 | Funnel plot of maternal occupational exposure to solvents and cleft lip with or without cleft palate in offspring



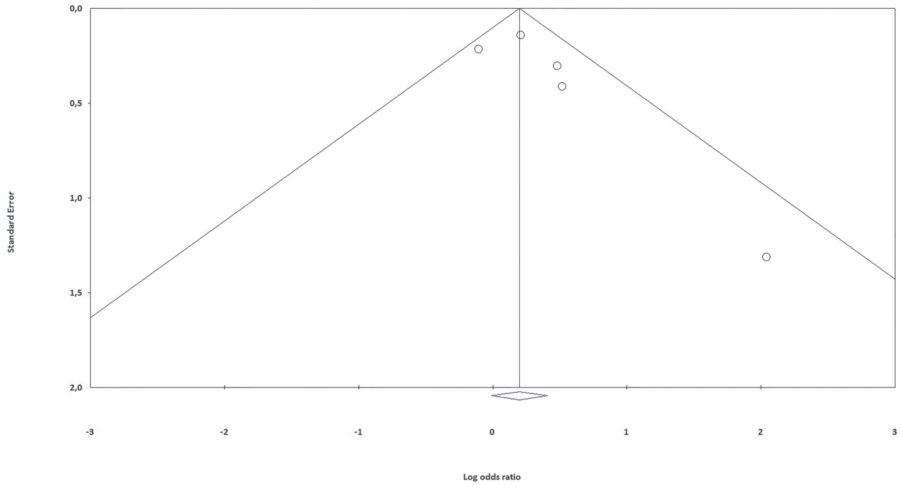
p-value = 0.10

Supplementary Figure 16 | Forest plot of maternal occupational exposure to solvents and risk of cleft palate in offspring



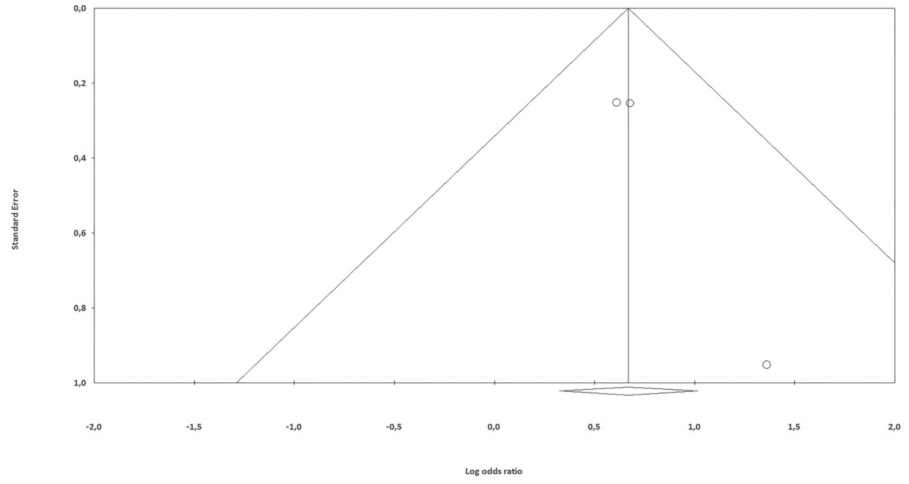
Heterogeneity: $\chi^2 = 5.42$, df = 4, $P = 0.25$, $I^2 = 26\%$

Supplementary Figure 17 | Funnel plot of maternal occupational exposure to solvents and risk of cleft palate in offspring



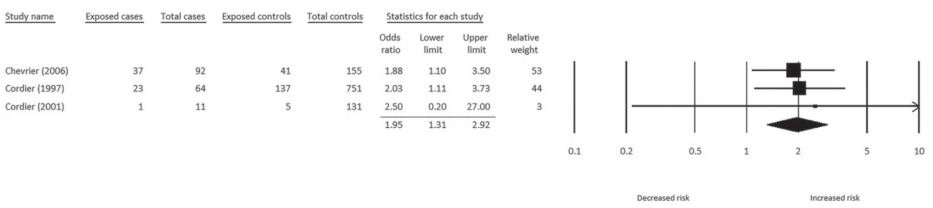
p-value = 0.11

Supplementary Figure 18 | Funnel plot of maternal occupational exposure to glycol ethers and risk of oral clefts in offspring



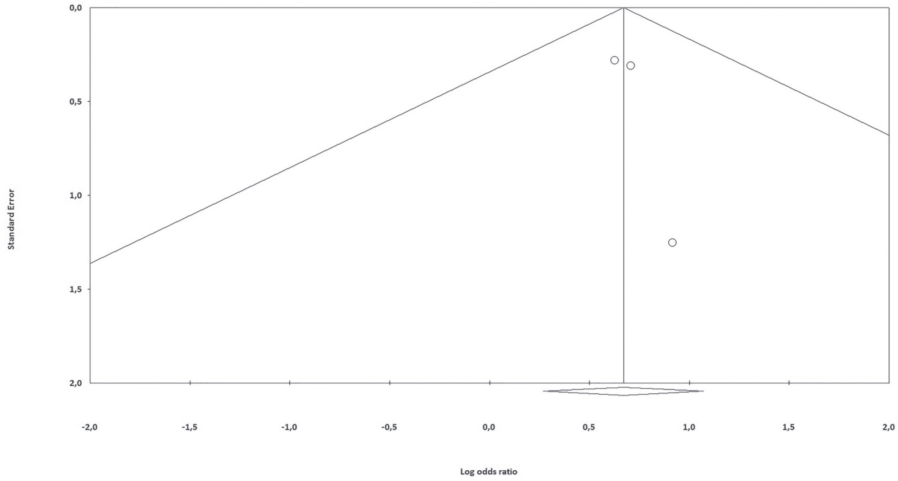
p-value = 0.08

Supplementary Figure 19 | Forest plot of maternal occupational exposure to glycol ethers and risk of cleft lip with or without cleft palate in offspring



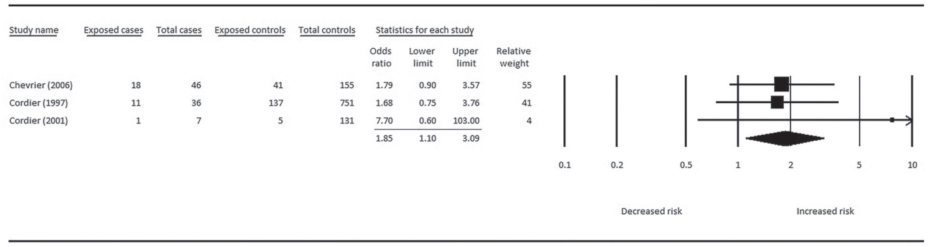
Heterogeneity: $\chi^2 = 0.08$, df = 2, $P = 0.96$, $I^2 = 0\%$

Supplementary Figure 20 | Funnel plot of maternal occupational exposure to glycol ethers and risk of cleft lip with or without cleft palate in offspring



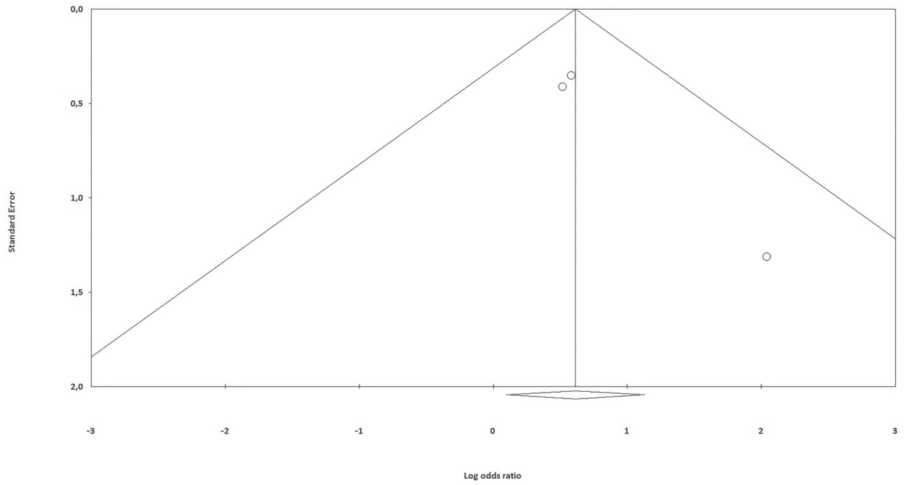
p-value = 0.22

Supplementary Figure 21 | Forest plot of maternal occupational exposure to glycol ethers and risk of cleft palate in offspring



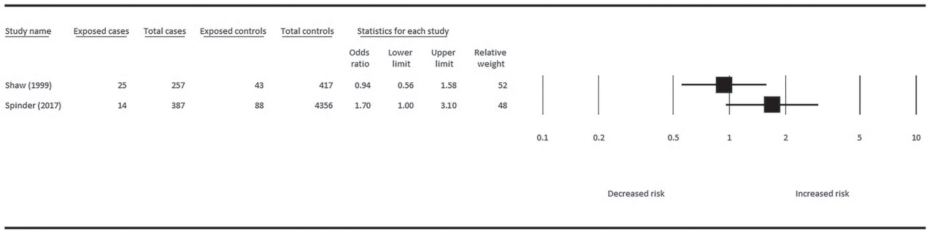
Heterogeneity: $\chi^2 = 1.25$, df = 2, $P = 0.54$, $I^2 = 0\%$

Supplementary Figure 22 | Funnel plot of maternal occupational exposure to glycol ethers and risk of cleft palate in offspring



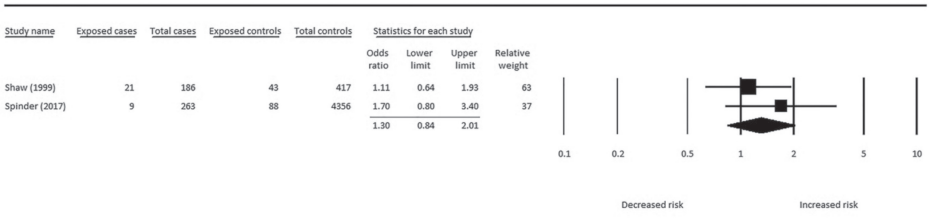
p-value = 0.08

Supplementary Figure 23 | Forest plot of maternal occupational exposure to pesticides and risk of oral clefts in offspring



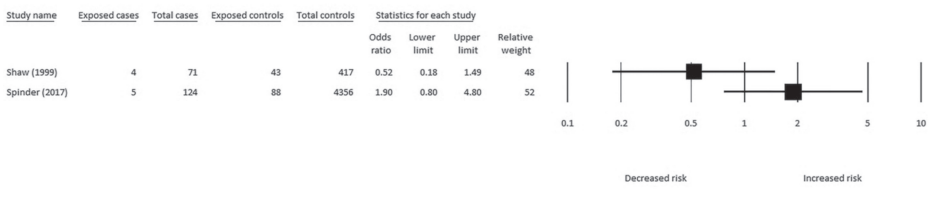
Heterogeneity: $\chi^2 = 2.31$, df = 1, $P = 0.13$, $I^2 = 57\%$

Supplementary Figure 24 | Forest plot of maternal occupational exposure to pesticides and risk of cleft lip with or without cleft palate in offspring



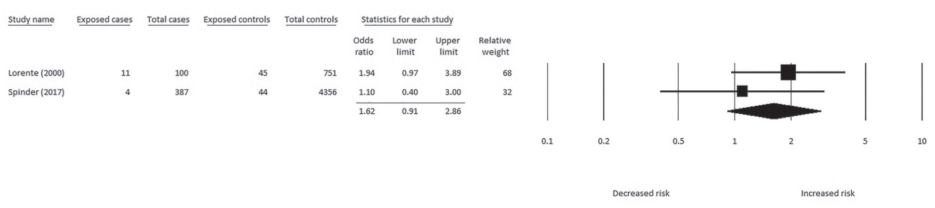
Heterogeneity: $\chi^2 = 0.85$, df = 1, $P = 0.36$, $I^2 = 0\%$

Supplementary Figure 25 | Forest plot of maternal occupational exposure to pesticides and risk of cleft palate in offspring



Heterogeneity: $\chi^2 = 3.37$, df = 1, $P = 0.07$, $I^2 = 70\%$

Supplementary Figure 26 | Forest plot of maternal occupational exposure to metals and risk of oral clefts in offspring

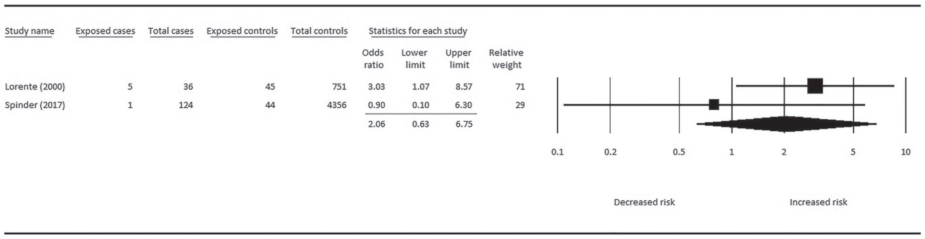


Heterogeneity: $\chi^2 = 0.82$, df = 1, $P = 0.36$, $I^2 = 0\%$

Supplementary Figure 27 | Forest plot of maternal occupational exposure to metals and risk of cleft lip with or without cleft palate in offspring

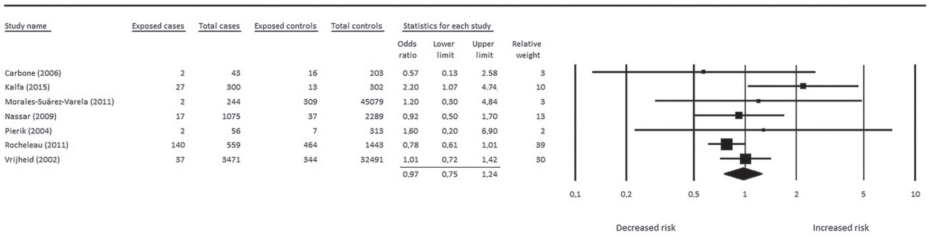
Heterogeneity: $\chi^2 = 0.18$, df = 1, $P = 0.67$, $I^2 = 0\%$

Supplementary Figure 28 | Forest plot of maternal occupational exposure to metals and risk of cleft palate in offspring



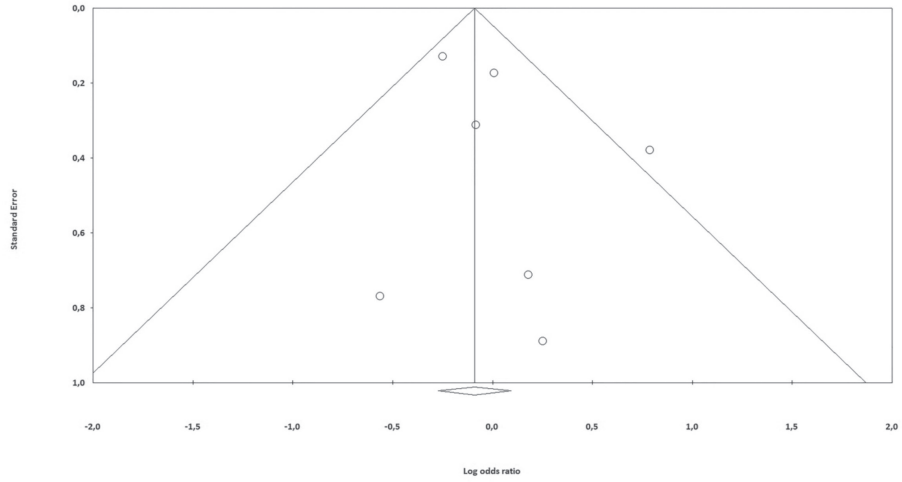
Heterogeneity: $\chi^2 = 1.36$, df = 1, $P = 0.24$, $I^2 = 26\%$

Supplementary Figure 29 | Forest plot of maternal occupational exposure to pesticides and risk of hypospadias in offspring



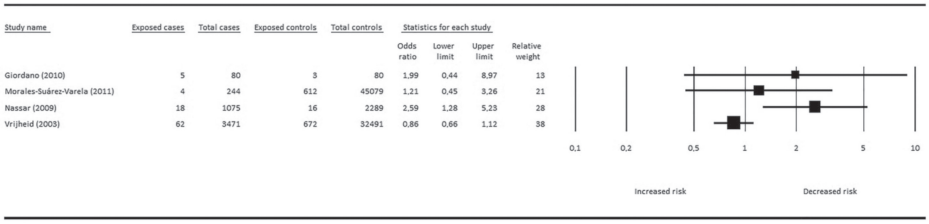
Heterogeneity: $\chi^2 = 7.85$, df = 6, $P = 0.25$, $I^2 = 24\%$

Supplementary Figure 30 | Funnel plot of maternal occupational exposure to pesticides and risk of hypospadias in offspring



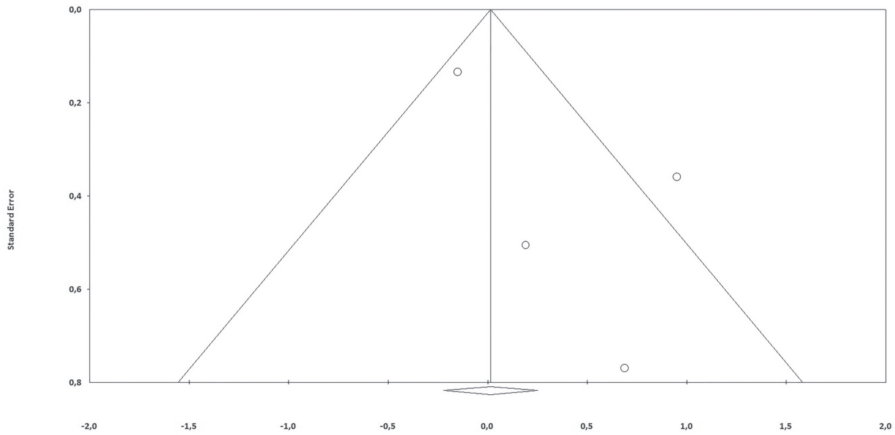
p-value = 0.17

Supplementary Figure 31 | Forest plot of maternal occupational exposure to metals and risk of hypospadias in offspring



Heterogeneity: $\chi^2 = 9.20$, df = 3, $P = 0.03$, $I^2 = 67\%$

Supplementary Figure 32 | Funnel plot of maternal occupational exposure to metals and risk of hypospadias in offspring



p-value = 0.13

CHAPTER 3

Maternal occupational exposure and oral clefts in offspring

Nynke Spinder
Jorieke E.H. Bergman
H. Marike Boezen
Roel C.H. Vermeulen
Hans Kromhout
Hermien E.K. de Walle

Environmental Health. 2017 Aug 4;16(1). 83.

ABSTRACT

Background Previous studies suggest that periconceptional maternal occupational exposure to solvents and pesticides increase the risk of oral clefts in the offspring. Less is known about the effect of occupational exposure to metals, dust, and gases and fumes on development of oral clefts.

Methods This case-malformed control study used data from a population-based birth defects registry (Eurocat)of children and foetuses born in the Northern Netherlands between 1997 and 2013. Cases were defined as nonsyndromic oral clefts. The first control group had chromosomal/monogenic defects, and the second control group was defined as non-chromosomal/non-monogenic malformed controls. Maternal occupational exposure was estimated through linkage of mothers' occupation with a community-based Job Exposure Matrix (JEM). Multivariate logistic regression was used to estimate the effect of occupational exposures. Odds ratios were adjusted (aORs) for relevant confounders.

Results A total of 387 cases, 1135 chromosomal and 4352 non-chromosomal malformed controls were included in this study. Prevalence of maternal occupational exposures to all agents was 43.9% and 41.0%/37.7% among cases and controls, respectively. Oral clefts had significantly increased ORs of maternal occupational exposure to pesticides (aOR 1.7, 95% confidence interval [CI] 1.0–3.1) and dust (aOR 1.3, 95%CI 1.1–1.6) when using nonchromosomal controls. Subgroup analysis for CL(P) stratified by gender showed a significantly increased risk for male infants exposed to 'other solvents' and exposure to mineral dust for female infants.

Conclusion Our study showed that maternal occupational exposure to pesticides and dust are risk factors for oral clefts in the offspring. Larger studies are needed to confirm this finding.

BACKGROUND

Oral clefts are one of the most common congenital anomalies in the Netherlands with a prevalence of 2.1 per 1000 live births [1]. Oral clefts are complex malformations that result from failure of fusion of the lip or palate. Because of different developmental origins, oral clefts can be classified as cleft palate (CP) or cleft lip with or without palate (CL(P)). Oral clefts have a large impact on the affected individuals, their parents and on the community in terms of physical and emotional wellbeing, and medical costs [2]. The aetiology of oral clefts is not fully understood, but involves genetic as well as environmental factors. Several environmental factors during pregnancy have been associated with an increased risk of oral clefts in the offspring, including maternal smoking [3], maternal alcohol consumption [4] and high maternal pre-pregnancy body mass index (BMI) (>30 kg/m2) [5-7]. There is no consensus on whether folic acid is protective or might be a risk factor for oral clefts [8].

Participation of Dutch women in the labour market has increased substantially over the last two decades [9]. Therefore, it is important to examine exposure to various teratogenic factors in the workplace. Large population based case-control studies suggest a relationship between exposure to organic solvents and oral clefts [10-17], whereas one other study did not find a higher risk of oral clefts in the offspring after maternal occupational exposure to solvents [18].

Several studies have investigated maternal occupational exposure to pesticides and risk of oral clefts in the offspring. Romitti et al. performed a meta-analysis and concluded that maternal exposure to pesticides in general is associated with a small increased risk of oral clefts in the offspring [19]. More recently, Yang et al. assessed residential exposure to specific agricultural pesticides in an area with high rates of pesticide use and concluded that there was a positive relationship between herbicide exposure and oral clefts, especially among female infants [20].

There is one previous study that suggested an association between maternal occupational exposure to metals and oral clefts in the offspring [21]. As far as we know, there is no literature concerning occupational exposure to mineral and organic dust, and gases and fumes in relation to the occurrence of oral clefts. However, since these exposures often occur in the same workplace as exposure to solvents and pesticides, these exposures were also taken into account in this study.

The objective of this case-malformed control study was to examine the association between maternal occupational exposure to, in particular solvents and pesticides, but also

to metals, mineral and organic dusts, and gases and fumes during the periconceptional period and risk of oral clefts in the offspring.

METHODS

Study design and population

To examine the possible association between maternal occupational exposure and oral clefts in the offspring a case-malformed control study was performed. Cases and malformed controls were selected from the European Concerted Action on Congenital Anomalies and Twins Northern Netherlands (Eurocat NNL). This population-based registry has been monitoring congenital anomalies in about 18,000 births annually in the provinces of Groningen, Friesland and Drenthe since 1981. In addition to live births (up to 10 years of age at notification), stillbirths, miscarriages and terminated pregnancies because of a congenital anomaly, are registered in the database. Children and foetuses are only registered in Eurocat NNL after parents give informed consent. In general, the informed consent rate is around 80% for all types of congenital anomalies. Coding and classification of congenital anomalies are performed according to Eurocat guidelines [22]. In this study, Eurocat NNL data of children and foetuses born from 1997 until 2013 was used.

Data collection

Since 1997, parents have been asked to complete a written questionnaire to supply information about the pregnancy. The questionnaire includes a question about maternal occupation and the workplace (e.g. the company where the mother worked) at the beginning of the pregnancy. In addition, information is gathered concerning medical history, demographic characteristics and maternal pre-pregnancy weight and height. For smoking habits, alcohol consumption, and the use of medication, information is gathered from three months before pregnancy until the end of pregnancy. After parental consent, data on prescribed medication is retrieved from the pharmacy. Ambiguities in the questionnaire, actual use of medication and for which period it was used, were verified in a telephone interview with the mother.

Definition of cases and controls

Cases were defined as non-syndromic clefts, either occurring isolated or together with other major congenital anomalies. Children with a Pierre Robin sequence were included in the case group. International Classification of Diseases 9th revision (ICD-9, 749) was used

for births up until 2001 and the ICD-10 classification (Q35-Q37) was used for births since 2002. A total of 679 cases with an oral cleft were selected for this study. Cases with a cleft that were also labelled as having a chromosomal or monogenic disorder were excluded (n = 89), because these clefts may be part of that specific syndrome. Additionally, cases with anencephaly, arhinencephaly and holoprosencephaly were excluded (n = 9) because these anomalies are often associated with oral clefts. In total, 95 cases (14%) were excluded because mothers' occupation was unknown (e.g. the questionnaire was not returned). In this study only mothers with a paid job were included, which led to an exclusion of 99 cases (e.g. housewives).

Non-malformed children are not registered in the Eurocat database. Infants and foetuses born with chromosomal/monogenic disorders, not accompanied by oral clefts, were used as controls, because the aetiology of these malformations is known. In total, 1764 chromosomal controls were selected for this study. We excluded 357 controls (20%) because mothers' occupation was unknown and another 272 controls were excluded because their mothers had no paid job. Hereafter we refer to this group as chromosomal controls.

Analyses were performed with a second control group, because chromosomal controls can introduce bias through higher maternal age. This second control group is defined as all other babies/foetuses registered in Eurocat with non-chromosomal/non-monogenic disorders, and no malformation accompanied by an oral cleft. A total of 6847 babies/foetuses were selected for the non-chromosomal malformed control group. Because mothers' occupation was unknown, 1626 controls (24%) were excluded. Furthermore, 869 controls were excluded because mother had no paid job. Hereafter we refer to this group as non-chromosomal controls. This resulted in a total of 387 cases, 1135 chromosomal controls and 4352 non-chromosomal controls. Cases were further subdivided in a group of CP (n = 124) and a group of CL(P) (n = 263).

Exposure assessment

A community-based JEM (ALOHA+ JEM) is applied to translate self-reported information about mothers' occupation during the periconceptional period (three months before conception through the first trimester) into occupational exposures to solvents, pesticides, metals and more generic categories like mineral and organic dust, and gases and fumes. The ALOHA+ JEM is built specifically for use in community-based studies [23]. Given that

specific occupational exposures are relatively rare in the general population, specificity in exposure assignment was preferred over sensitivity when elaborating the ALOHA+ JEM [24].

Jobs were coded by two of the authors (NS and HK) into the International Standard Classification of Occupations 1988 (ISCO88) without knowledge of case/control status [25]. The ALOHA+ JEM assigned occupational exposure to solvents (aromatic, chlorinated and other [e.g. alkanes, alcohols, and esters]), pesticides (fungicides, herbicides and insecticides), metals, dust (organic and mineral), and gases and fumes. Based on the mothers' occupation, the JEM assigned no (0), low (1) or high (2) exposure to solvents, pesticides, metals, dust, and gases and fumes. For mothers who had two or more jobs with different exposures, the highest exposure category was selected.

Variable definition

Potential confounders applied in our analyses were child sex (boy or girl), number of babies/foetuses delivered (1 or ≥2), previous births (0, 1 or ≥2 births), maternal age at delivery (15–19, 20–24, 25–29, 30–39, ≥40 years old), maternal BMI (underweight [<18.5 kg/m2], normal [18.5–25 kg/m2], overweight [25–30 kg/m2], obese [>30 kg/m2]), maternal education level (low [primary school, lower vocational education, pre-vocational education], middle [secondary vocational education, general secondary education or pre-university education] or high [higher professional education or academic education]), maternal smoking (no, yes/some period during pregnancy), maternal alcohol use during pregnancy (no, yes/ some period during pregnancy), folic acid use (no/wrong period, yes/periconceptional period [400 µg folic acid/ day from 4 weeks before until 8 weeks after conception [26]), fertility problems (no, yes [self-reported fertility problems or fertility treatment]) and positive family history (yes/no). A positive family history means a first degree family member with the same condition as the baby/foetus under study, e.g. if a child has an oral cleft, the family history is positive when a first degree family member has an oral cleft as well.

Statistical analyses

The associations between specific maternal occupational exposures and oral clefts were assessed using univariate and multivariate logistic regression models to estimate crude odds ratios (OR) and adjusted ORs. We adjusted multivariate models for potential confounders, based on significance using Chi Square tests. Confounders for the analyses with chromosomal controls were child sex, maternal age at delivery, pre-pregnancy BMI, education level, smoking and alcohol use during pregnancy, and family history.

Analyses with non-chromosomal controls were corrected for child sex and previous births as confounders. Separate subgroup analyses were conducted for CP and CL(P) alone compared with both control groups.

From literature is known that the prevalence of CL(P) is higher among male infants. Therefore, an additional analysis was performed stratified by child's gender. Due to the small number of mothers with high exposure, low and high exposure were merged into one 'any exposure' group for all types of occupational exposures. Additionally, for specific exposure categories with a high prevalence of exposed cases, it was possible to evaluate no, low, and high exposure categories separately. P-values of <0.05 were considered statistically significant. Statistical Package for the Social Sciences version 22 (SPSS V22) was used to perform all analyses.

RESULTS

The baseline characteristics of 387 cases, 1135 chromosomal controls and 4352 non-chromosomal controls are presented in Table 1. Among cases there was a significant excess of males compared to chromosomal controls. Case mothers had a younger age at delivery, a higher BMI and their education level was lower. Furthermore, they smoked more often, used alcohol less often, and had less often a positive family history. The significant differences in baseline characteristics between oral clefts and chromosomal controls apply as well when CL(P) and chromosomal controls were compared, except for pre-pregnancy BMI. There were no significant differences in baseline characteristics between CP and chromosomal controls.

Table 1| Baseline characteristics of cases (all oral clefts, cleft palate (CP), cleft lip with/without cleft palate CL(P)) compared with two malformed control groups.

	Chromosomal controls[a]		**Non-chromosomal controls[b]**		**All oral cleft**				**CP**				**CL(P)**			
	n	%	n	%	n	%	p-value[c]	p-value[d]	n	%	p-value[c]	p-value[d]	n	%	p-value[c]	p-value[d]
	1135		4352		387				124				263			
Child sex							**<0.001**	0.21			0.83	0.43			**<0.001**	**0.02**
Boy	547	48.2	2373	54.5	227	58.7			61	49.2			166	63.1		
Girl	588	51.8	1970	45.3	160	41.3			63	50.8			97	36.9		
Unknown	0		9		0				0				0			
Number of babies/foetuses delivered							0.31	0.98			0.09	0.30			0.86	0.50
1	1089	96.0	4104	94.8	366	94.8			115	92.7			251	95.8		
>1	45	4.0	223	5.2	20	5.2			9	7.3			11	4.2		
Unknown	1		25		1				0				1			
Previous births							0.24	**0.01**			0.60	0.11			0.36	0.05
0	476	42.3	2313	53.2	180	46.6			57	46.0			123	46.9		
1	461	40.9	461	34.6	141	36.5			45	36.3			96	36.6		
≥2	189	16.8	189	12.1	65	16.8			22	17.7			43	16.4		
Unknown	9		8			1			0				1			
Maternal age at delivery							**<0.001**	0.66			0.09	0.40			**<0.001**	0.66
15-19	4	0.4	14	0.3	2	0.5			0				2	0.8		
20-24	55	4.8	369	8.5	24	6.2			8	6.5			16	6.1		
25-29	299	26.3	1458	33.5	132	34.3			43	35.0			89	34.0		
30-34	407	35.9	1770	40.7	154	40.0			47	38.2			107	40.8		
35-39	284	25.0	651	15.0	63	16.4			19	15.4			44	16.8		
>40	86	7.6	86	2.0	10	2.6			6	4.9			4	1.5		
Unknown	0		0		2				1				1			

Table 1. Continued

	Chromosomal controls[a]		Non-chromosomal controls[b]		All oral cleft				CP				CL(P)			
	n	%	n	%	n	%	p-value[c]	p-value[d]	n	%	p-value[c]	p-value[d]	n	%	p-value[c]	p-value[d]
	1135		4352		387				124				263			
Pre-pregnancy BMI (kg/m^2)							**0.03**	0.21			0.19	0.49			0.09	0.29
<18.5	30	2.8	112	2.7	12	3.2			2	1.7			10	3.9		
18.5-25	721	66.3	2713	64.6	224	59.1			71	59.2			153	59.1		
25-30	258	23.7	975	23.2	101	26.6			33	27.5			68	26.3		
>30	78	7.2	402	9.6	42	11.1			14	11.7			28	10.8		
Unknown	48		150		8				4				4			
Education level							**0.01**	0.84			0.59	0.72			**0.01**	0.56
Low	151	13.8	545	12.8	51	13.5			15	12.4			36	14.0		
Middle	462	42.2	2137	50.3	193	51.1			57	47.1			136	52.9		
High	481	44.0	1568	36.9	134	35.4			49	40.5			85	33.1		
Unknown	41		102		9				3				6			
Smoking during pregnancy							**0.02**	0.47			0.72	0.49			**0.01**	0.18
No	89	81.0	3321	77.0	291	75.4			98	79.7			193	73.4		
Yes	210	19.0	992	23.0	95	24.6			25	20.3			70	26.6		
Unknown	28		39		1				1				0			
Alcohol during pregnancy							**0.02**	0.16			0.25	0.62			**0.03**	0.17
No	811	73.3	3276	76.1	306	79.3			96	78.0			210	79.8		
Yes	296	26.7	1029	23.9	80	20.7			27	22.0			53	20.2		
Unknown	28		47		1				1				0			

Table 1. Continued

	Chromosomal controls[a]		**Non-chromosomal controls[b]**		**All oral cleft**		**CP**				**CL(P)**			
	n	%	n	%	n	%	p-value[c]	p-value[d]	n	%	p-value[c]	p-value[d]	n	%	p-value[c]	p-value[d]
	1135		4352		387				124				263			
Folic acid use							0.64	0.84			0.67	0.52			0.34	0.49
No	224	21.3	844	20.5	77	20.1			28	23.0			49	18.8		
Yes	830	78.7	3265	79.5	306	79.9			94	77.0			212	81.2		
Unknown	81		243		4				2				2			
Fertility problems							0.39	0.29			0.73	0.11			0.19	0.84
No	889	81.0	3634	85.0	316	82.9			98	79.7			218	84.5		
Yes	209	19.0	643	15.0	65	17.1			25	20.3			40	15.5		
Unknown	37		75		6				1				5			
Positive family history[e]							**0.003**	0.08			0.17	0.60			**0.005**	0.07
No	960	85.0	3819	88.0	352	91.0			111	89.5			241	91.6		
Yes	170	15.0	523	12.0	35	9.0			13	10.5			22	8.4		
Unknown	4		10		0				0				0			

[a]=chromosomal/monogenic controls (not accompanied with oral clefts), [b]=non-chromosomal/non-monogenic malformed controls (not accompanied with oral clefts), [c]=p-value comparing cases with chromosomal controls, [d]=p-value comparing cases with non-chromosomal controls, [e]=positive family history when first degree family member has same condition as child under study. Bold values represent significant values (p<0.05).

None of these significant differences were observed when cases were compared with non-chromosomal controls, except the excess of males in the CL(P) group. Mothers with a child with an oral cleft had significantly more previous births. The prevalence of estimated occupational exposure to any of the agents considered was 43.9% among case mothers, 41.0% among mothers of chromosomal controls (Table 2), and 37.7% among nonchromosomal controls (Table 3). Prevalence of maternal exposure to solvents was similar among cases and controls. The most frequent type of solvent exposure was exposure to 'other solvents'. Mothers exposed to 'other solvents' were mainly working in healthcare. The prevalence of occupational exposure to pesticides was low, but was higher among cases than controls (3.6% versus 2.4% for chromosomal controls and 2.0% for non-chromosomal controls). Maternal occupational exposure to organic dust occurred most frequent, with case mothers being more often exposed to organic dust than chromosomal/non-chromosomal controls (36.7% versus 32.6%/29.6%). Mothers exposed to organic dust were working in e.g. healthcare or agriculture. Table 2 shows the adjusted ORs of maternal occupational exposure. The aORs for maternal occupational exposure to solvents, metals, dust, and gases and fumes did not increase significantly when using chromosomal controls. When using non-chromosomal controls, aORs increased significantly for maternal occupational exposure to pesticides and dust (Table 3). The highest aORs were found for fungicides and insecticides (aOR 2.0, 95%CI 1.1–3.7 and aOR 1.8, 95%CI 1.0–3.2, respectively). The aOR for dust, especially organic dust, increased significantly (aOR 1.3, 95%CI 1.1–1.7). The significant changes were also observed for organic dust in the CL(P) group. Additional analyses with CL(P) cases were performed stratified by child sex. The aOR for periconceptional exposure to 'other solvents' increased for male infants (aOR 1.5, 95%CI 1.1–2.1, data not shown in Table) using non-chromosomal controls. The aOR for occupational herbicide exposure in relation to CL(P) increased for female infants (aOR 3.8, 95%CI 1.1–13.4, data not shown in Table). However, this was only based on three exposed cases. Mineral dust exposure was associated with CL(P) for females as well (aOR 2.0, 95%CI 1.2-3.5, data not shown in Table). For exposure categories with high prevalence in this study ('other solvents', organic dust, and gases and fumes), additional analyses were performed for all three exposure intensity categories (no, low, and high). The number of high exposed cases was respectively 10, 11, and 4 cases. The aOR for cases with low exposure to 'other solvents' was 1.1 (95%CI 0.8–1.5), and increased to 1.5 (95%CI 0.8–3.0) for cases with high exposure (data not shown in Table). For occupational exposure to organic dust the same trend is observed. The aOR increased from 1.3 (95%CI 1.1–1.6) for low exposure, to 1.7 (95%CI 0.9–3.2) for high exposure (data not shown in Table). No trend

of increased is observed OR for occupational exposure to gases and fumes. However, all ORs did not increase significantly.

Table 2 | Prevalence exposures and association between periconceptional maternal occupational exposure and all oral clefts, cleft palate (CP), and cleft lip with/without cleft palate (CL(P)) using chromosomal/monogenic controls.

Exposure	Diagnosis	Prevalence exposure		Unadjusted		Adjusted[a]	
		n	%	OR	95% CI	OR	95 %CI
Any agent							
	Chromosomal control	465	41.0	Ref		Ref	
	All oral cleft	170	43.9	1.1	0.9 – 1.4	1.0	0.8 – 1.3
	CP	42	33.9	0.7	0.5 – 1.1	0.7	0.5 – 1.1
	CL(P)	128	48.7	1.4	1.0 – 1.8	1.2	0.9 – 1.6
Solvents							
	Chromosomal control	281	24.8	Ref			
	All oral cleft	103	26.6	1.1	0.8 – 1.4	1.0	0.8 – 1.4
	CP	29	23.4	0.9	0.6 – 1.4	0.9	0.6 – 1.4
	CL(P)	74	28.1	1.2	0.9 – 1.6	1.1	0.8 – 1.5
Aromatic solvents							
	Chromosomal control	50	4.4	Ref		Ref	
	All oral cleft	17	4.4	1.0	0.6 – 1.8	1.1	0.6 – 1.8
	CP	5	4.0	0.9	0.4 – 2.3	1.0	0.4 – 2.6
	CL(P)	12	4.6	1.0	0.5 – 2.0	1.1	0.5 – 2.0
Chlorinated solvents							
	Chromosomal control	53	4.7	Ref		Ref	
	All oral cleft	18	4.7	1.0	0.6 – 1.7	1.0	0.5 – 1.7
	CP	4	3.2	0.7	0.2 – 1.9	0.7	0.2 – 1.9
	CL(P)	14	5.3	1.1	0.6 – 2.1	1.1	0.6 – 2.0
Other solvents							
	Chromosomal control	263	23.2	Ref		Ref	
	All oral cleft	99	25.6	1.2	0.9 – 1.5	1.1	0.8 – 1.4
	CP	28	22.6	1.0	0.6 – 1.5	0.9	0.6 – 1.5
	CL(P)	71	27.0	1.2	0.9 – 1.7	1.2	0.8 – 1.6
Pesticides							
	Chromosomal control	27	2.4	Ref		Ref	
	All oral cleft	14	3.6	1.5	0.8 – 3.0	1.5	0.8 – 3.0
	CP	5	4.0	1.7	0.7 – 4.6	1.7	0.6 – 4.6
	CL(P)	9	3.4	1.5	0.7 – 3.1	1.4	0.6 – 3.1

Table 2. Continued

Exposure	Diagnosis	Prevalence exposure		Unadjusted		Adjusted[a]	
		n	%	OR	95% CI	OR	95 %CI
Fungicides							
	Chromosomal control	23	2.0	Ref		Ref	
	All oral cleft	13	3.4	1.7	0.8 – 3.4	1.7	0.8 – 3.5
	CP	5	4.0	2.0	0.8 – 5.4	2.1	0.7 – 5.7
	CL(P)	8	3.0	1.5	0.7 – 3.4	1.5	0.6 – 3.4
Herbicides							
	Chromosomal control	15	1.3	Ref		Ref	
	All oral cleft	6	1.6	1.2	0.5 – 3.1	1.2	0.4 – 3.1
	CP	1	0.8	0.6	0.1 – 4 6	0.6	0.1 – 4.6
	CL(P)	5	1.9	1.4	0.5 – 4.0	1.3	0.5 – 3.9
Insecticides							
	Chromosomal control	25	2.2	Ref		Ref	
	All oral cleft	14	3.6	1.7	0.9 – 3.2	1.7	0.8 – 3.3
	CP	5	4.0	1.9	0.7 – 5.0	1.8	0.7 – 5.0
	CL(P)	9	3.4	1.6	0.7 – 3.4	1.5	0.7 – 3.3
Heavy metals							
	Chromosomal control	14	1.3	Ref		Ref	
	All oral cleft	4	1.0	0.8	0.3 – 2.6	0.6	0.2 – 2.3
	CP	1	0.8	0.7	0.1 – 5.0	0.6	0.1 – 5.0
	CL(P)	3	1.1	0.9	0.3 – 3.2	0.8	0.2 – 3.1
Dust							
	Chromosomal control	385	33.9	Ref		Ref	
	All oral cleft	146	37.7	1.2	0.9 – 1.5	1.1	0.9 – 1.4
	CP	37	28.8	0.8	0.5 – 1.2	0.8	0.5 – 1.2
	CL(P)	109	41.4	1.3	0.9 – 1.7	1.3	0.9 – 1.7
Organic dust							
	Chromosomal control	370	32.6	Ref		Ref	
	All oral cleft	142	36.7	1.2	0.9 – 1.5	1.2	0.9 – 1.4
	CP	36	29.0	0.8	0.6 – 1.3	0.8	0.5 – 1.2
	CL(P)	106	40.3	1.4	1.1 – 1.8	1.3	0.9 – 1.7
Mineral dust							
	Chromosomal control	111	9.8	Ref		Ref	
	All oral cleft	40	10.3	1.1	0.7 – 1.6	1.1	0.7 – 1.6
	CP	9	7.3	0.7	0.4 – 1.5	0.8	0.4 – 1.6
	CL(P)	31	11.8	1.2	0.8 – 1.9	1.2	0.8 – 2.0

Table 2. Continued

Exposure	Diagnosis	Prevalence exposure		Unadjusted		Adjusted[a]	
		n	%	OR	95% CI	OR	95 %CI
Gases and fumes							
	Chromosomal control	353	31.1	Ref		Ref	
	All oral cleft	126	32.6	1.1	0.8 – 1.4	1.0	0.8 – 1.3
	CP	29	23.4	0.7	0.4 – 1.0	0.6	0.4 – 1.0
	CL(P)	97	36.9	1.3	1.0 – 1.7	1.2	0.9 – 1.6

[a]=Odds ratio adjusted for child sex, maternal age at delivery, pre-pregnancy body mass index, education level, smoking and alcohol use during pregnancy, and family history.

Table 3 | Prevalence exposures and association between periconceptional maternal occupational exposure and all oral clefts, cleft palate (CP), and cleft lip with/without cleft palate (CL(P)) using non-chromosomal/non-monogenic malformed controls.

Exposure	Diagnosis	Prevalence exposure		Unadjusted		Adjusted[a]	
		n	%	OR	95% CI	OR	95 %CI
Any agent							
	Chromosomal control	1642	37.7	Ref		Ref	
	All oral cleft	170	43.9	**1.3**	**1.0 – 1.6**	**1.3**	**1.0 – 1.6**
	CP	42	33.9	0.8	0.6 – 1.2	0.8	0.6 – 1.2
	CL(P)	128	48.7	**1.6**	**1.2 – 2.0**	**1.5**	**1.2 – 2.0**
Solvents							
	Chromosomal control	1075	24.7	Ref			
	All oral cleft	103	26.6	1.1	0.9 – 1.4	1.1	0.9 – 1.4
	CP	29	23.4	0.9	0.6 – 1.4	0.9	0.6 – 1.4
	CL(P)	74	28.1	1.2	0.9 – 1.6	1.2	0.9 – 1.6
Aromatic solvents							
	Chromosomal control	140	3.2	Ref		Ref	
	All oral cleft	17	4.4	1.4	0.8 – 2.3	1.4	0.8 – 2.3
	CP	5	4.0	1.3	0.5 – 3.1	1.3	0.5 – 3.1
	CL(P)	12	4.6	1.4	0.8 – 2.6	1.5	0.8 – 2.7
Chlorinated solvents							
	Chromosomal control	190	4.4	Ref		Ref	
	All oral cleft	18	4.7	1.1	0.7 – 1.8	1.1	0.7 – 1.8
	CP	4	3.2	0.7	0.3 – 2.0	0.7	0.3 – 2.0
	CL(P)	14	5.3	1.2	0.7 – 2.2	1.3	0.7 – 2.2
Other solvents							
	Chromosomal control	1042	23.9	Ref		Ref	
	All oral cleft	99	25.6	1.1	0.9 – 1.4	1.1	0.9 – 1.4
	CP	28	22.6	0.9	0.6 – 1.4	0.9	0.6 – 1.4
	CL(P)	71	27.0	1.2	0.9 – 1.6	1.2	0.9 – 1.6

Table 3. Continued

Exposure	Diagnosis	Prevalence exposure		Unadjusted		Adjusted[a]	
		n	%	OR	95% CI	OR	95 %CI
Pesticides							
	Chromosomal control	88	2.0	Ref		Ref	
	All oral cleft	14	3.6	**1.8**	**1.0 – 3.2**	**1.7**	**1.0 – 3.1**
	CP	5	4.0	2.0	0.8 – 5.1	1.9	0.8 – 4.8
	CL(P)	9	3.4	1.7	0.9 – 3.4	1.7	0.8 – 3.4
Fungicides							
	Chromosomal control	70	1.6	Ref		Ref	
	All oral cleft	13	3.4	**2.1**	**1.2 – 3.9**	**2.0**	**1.1 – 3.7**
	CP	5	4.0	**2.6**	**1.0 – 6.5**	2.4	0.9 – 6.0
	CL(P)	8	3.0	1.8	0.9 – 3.6	1.9	0.9 – 4.0
Herbicides							
	Chromosomal control	36	0.8	Ref		Ref	
	All oral cleft	6	1.6	1.9	0.8 – 4.5	1.8	0.8 – 4.4
	CP	1	0.8	1.0	0.1 – 7 2	0.9	0.1 – 7.0
	CL(P)	5	1.9	2.3	0.9 – 6.0	2.3	0.9 – 5.9
Insecticides							
	Chromosomal control	84	1.9	Ref		Ref	
	All oral cleft	14	3.6	**1.9**	**1.1 – 3.4**	**1.8**	**1.0 – 3.2**
	CP	5	4.0	2.1	0.9 – 5.4	2.0	0.8 – 5.0
	CL(P)	9	3.4	1.8	0.9 – 3.6	1.7	0.9 – 3.5
Heavy metals							
	Chromosomal control	44	1.0	Ref		Ref	
	All oral cleft	4	1.0	1.0	0.4 – 2.9	1.1	0.4 – 3.0
	CP	1	0.8	0.8	0.1 – 5.8	0.9	0.1 – 6.3
	CL(P)	3	1.1	1.1	0.3 – 3.7	1.2	0.4 – 3.8
Dust							
	Chromosomal control	1346	30.9	Ref		Ref	
	All oral cleft	146	37.7	**1.4**	**1.1 – 1.7**	**1.3**	**1.1 – 1.6**
	CP	37	28.8	1.0	0.6 – 1.4	0.9	0.6 – 1.4
	CL(P)	109	41.4	**1.6**	**1.2 – 2.0**	**1.5**	**1.2 – 2.0**
Organic dust							
	Chromosomal control	1288	29.6	Ref		Ref	
	All oral cleft	142	36.7	**1.4**	**1.1 – 1.7**	**1.3**	**1.1 – 1.7**
	CP	36	29.0	1.0	0.7 – 1.4	1.0	0.6 – 1.4
	CL(P)	106	40.3	**1.6**	**1.2 – 2.1**	**1.6**	**1.2 – 2.0**

Table 3. Continued

Exposure	Diagnosis	Prevalence exposure		Unadjusted		Adjusted[a]	
		n	%	OR	95% CI	OR	95 %CI
Mineral dust							
	Chromosomal control	396	9.1	Ref		Ref	
	All oral cleft	40	10.3	1.2	0.8 – 1.6	1.1	0.8 – 1.6
	CP	9	7.3	0.8	0.4 – 1.6	0.8	0.4 – 1.5
	CL(P)	31	11.8	1.3	0.9 – 2.0	1.3	0.9 – 1.9
Gases and fumes							
	Chromosomal control	1521	34.9	Ref		Ref	
	All oral cleft	126	32.6	0.8	0.7 – 1.1	0.9	0.7 – 1.1
	CP	29	23.4	**0.6**	**0.4 – 0.9**	**0.6**	**0.4 – 0.9**
	CL(P)	97	36.9	1.1	0.8 – 1.4	1.1	0.8 – 1.4

[a]=Odds ratio adjusted for child sex, maternal age at delivery, pre-pregnancy body mass index, education level, smoking and alcohol use during pregnancy, and family history.

DISCUSSION

Results from this population-based case-malformed control study indicate an effect for maternal periconceptional occupational exposure to fungicides, insecticides, and organic dust on the risk of oral clefts in the offspring. Male infants have an increased risk on CL(P) when mothers are occupational exposed to 'other solvents'. Females have an increased on CL(P) when mothers are exposed to mineral dust. This study shows overall no increased risk of clefts in the offspring when mothers are periconceptionally occupational exposed to solvents, metals, and gases and fumes.

The association between maternal pesticide exposure and oral clefts in the offspring is described previously. A meta-analysis from 2007, that examined the association between occupational exposure to pesticides during pregnancy and oral clefts, showed a significant increased risk of oral clefts (OR 1.37, 95%CI 1.04– 1.81) [19]. This is comparable to our study, where we find slightly higher OR of 1.7, 95%CI 1.0–3.1. Most mothers exposed to pesticides in our study were working in agriculture. A Finnish study examined the association between working in agriculture and oral clefts in the offspring [27]. They found a comparable increased OR of oral clefts in the offspring among mothers working in agriculture during the first trimester of their pregnancy (OR 1.9, 95%CI 1.1–3.5).

Furthermore, we observed an association between maternal exposure to dust and oral clefts in the offspring. Despite the fact that occupational exposure to dust is common

at the workplace, no studies are known about the relation between occupational dust exposure and congenital anomalies in the offspring.

In our study we found no association between maternal occupational exposure to solvents and oral clefts in the main analyses. However, in the additional analyses an association is found between maternal occupational exposure to 'other solvents' and CL(P) in male infants only. Our finding is in line with one study from the USA that reported no association [18], but it is in contrast with multiple studies published since 2000 that did report an association between maternal occupational exposure to solvents and oral clefts [10-16]. Most of these studies have been performed in France and the USA and used occupational hygienists, who assessed exposure to specific solvents case-by-case based on detailed standardized interviews in which mothers were asked about job titles and descriptions of the job. The method of classifying occupational exposure by industrial hygienists is more specific and accurate than use of a JEM. However, there is a prospective study, using self-reported exposure assessment as well as a JEM, which reports a significant increased risk of oral clefts in the offspring for mothers exposed to solvents [12]. Inconsistencies could also be due to different definitions of solvent exposure.

We found no significant association between maternal occupational exposure to metals and oral clefts, whereas the study of Hao et al. [21] did find a significant association (OR 5.67, 95%CI 1.34–24.09). In our study the prevalence of exposure was very low compared to the Chinese study (0.8% in our CP group versus 8.8% in Hao et al.). No other studies have investigated metal exposure in relation to oral clefts.

Finally, we observed no association between maternal occupational exposure to gases and fumes, which we analysed because these are often co-exposures in women exposed to pesticides, solvents and metals.

Strengths and limitations

A major strength of this study is the use of data from the population-based Eurocat registry. Ascertainment of oral cleft cases by Eurocat NNL was virtually complete for birth years 1997–2009, with a consent rate for registration of over 90% [8]. Data in the Eurocat NNL database are of high quality and congenital anomalies are classified according to high standards and ICD codes. This made it possible to accurately distinguish between isolated clefts, clefts occurring together with other major congenital anomalies and syndromic clefts. Moreover, because both cases and both control groups had anomalies, recall bias is not expected to play a role in our study design.

Another strength is the use of the ALOHA+ JEM. The benefit of using a JEM is that it avoids recall bias since the mother is not directly asked about her occupational exposure during pregnancy. Besides, results in occupational exposure estimates are that are less prone to differential misclassification of exposure compared to self-reported exposures [24,28].

The Eurocat NNL questionnaire includes questions about job title and workplace during pregnancy, but did not include questions about the actual job tasks that were performed. It is therefore possible that women avoided certain activities during the periconceptional period in order to decrease exposure to potential teratogenic agents. Their actual exposure could therefore have been lower or absent from what was assigned by the JEM based on their job. Another limitation of using a JEM, compared to expert assessment, is that JEMs have often low sensitivity. Partly, this low sensitivity is due to the variability in exposure across time which is not taken into account by the JEM [29].

In our study a relatively low numbers of cases are exposed to pesticides. This has resulted in a lower power. Besides, our study could not address exposure intensity for all subcategories of exposure as assigned by the JEM (low or high exposure) separately in our analyses, due to the low numbers of highly exposed women. This precluded an exposure-response evaluation.

Finally, we used malformed controls and could therefore not compare with healthy children. It is known that occupational exposure to pesticides is possibly associated with chromosomal aberrations [30]. Furthermore, residential exposure to solvents or metals has been suggested to be associated with an increased risk of chromosomal anomalies in the offspring of older women [31]. Given our design, if these associations between occupational exposure and chromosomal anomalies would have been present, this would have resulted in attenuated risk estimates of maternal occupational exposures for the risk of oral clefts in the offspring.

Conclusion

Our study indicates that maternal periconceptional occupational exposure to pesticides and dust are risk factors for oral clefts, in particular exposure to fungicides, insecticides and organic dust is associated with an increased risk for cleft palate in the offspring. Occupational maternal exposure to 'other solvents' gives an increased risk of CL(P) in male offspring, whereas mineral dust is associated with CL(P) in female offspring. Exposure to solvents, metals, and gases and fumes are not shown to be associated with oral clefts in the offspring. More data are needed to identify whether the association between periconceptional occupational maternal solvents, pesticides, and dust exposure and cleft palate in the offspring is causal.

REFERENCES

1. Eurocat Nederland. Update: Actuele cijfers aangeboren aandoeningen 2013. Available from: http://www.rug.nl/research/genetics/eurocat/algemene-cijfers-tabel-2013-final.pdf. Updated 2014. Accessed 5/7, 2015.
2. Nidey N, Moreno Uribe LM, Marazita MM, Wehby GL. Psychosocial well-being of parents of children with oral clefts. Child Care Health Dev. 2015;42(1):42–50.
3. Leite M, Albieri V, Kjaer SK, Jensen A. Maternal smoking in pregnancy and risk for congenital malformations: Results of a danish register-based cohort study. Acta Obstet Gynecol Scand. 2014;93(8):825-834.
4. Romitti PA, Sun L, Honein MA, Reefhuis J, Correa A, Rasmussen SA. Maternal periconceptional alcohol consumption and risk of orofacial clefts. Am J Epidemiol. 2007;166(7):775-785.
5. Rankin J, Tennant PWG, Stothard KJ, Bythell M, Summerbell CD, Bell R. Maternal body mass index and congenital anomaly risk: A cohort study. Int J Obes. 2010;34:1371–80.
6. Stothard KJ, Tennant PW, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: A systematic review and meta-analysis. JAMA. 2009;301(6):636-650.
7. Kutbi H, Wehby GL, Moreno Uribe LM, et al. Maternal underweight and obesity and risk of orofacial clefts in a large international consortium of population-based studies. Int J Epidemiol. 2016:1–10.
8. Rozendaal AM, van Essen AJ, te Meerman GJ, et al. Periconceptional folic acid associated with an increased risk of oral clefts relative to non-folate related malformations in the Northern Netherlands: A population based case-control study. Eur J Epidemiol. 2013;28(11):875-887.
9. Centraal Bureau voor de Statistiek. Statistics Netherlands: Female labour participation stabilising following years of increase. Available from: http://www.cbs.nl/en-GB/menu/themas/dossiers/vrouwen-en-mannen/publicaties/artikelen/archief/2014/2014-4196-wm.htm. Updated 2014. Accessed 9/23, 2015.
10. Desrosiers TA, Lawson CC, Meyer RE, et al. Maternal occupational exposure to organic solvents during early pregnancy and risks of neural tube defects and orofacial clefts. Occupational and Environmental Medicine. 2012;69(7):493-499.
11. Langlois PH, Hoyt AT, Lupo PJ, et al. Maternal occupational exposure to polycyclic aromatic hydrocarbons and risk of oral cleft-affected pregnancies. Cleft Palate Craniofac J. 2013;50(3):337-346.
12. Garlantezec R, Monfort C, Rouget F, Cordier S. Maternal occupational exposure to solvents and congenital malformations: A prospective study in the general population. Occup Environ Med. 2009;66(7):456-463.
13. Lorente C, Cordier S, Bergeret A, et al. Maternal occupational risk factors for oral clefts. occupational exposure and congenital malformation working group. Scand J Work Environ Health. 2000;26(2):137-145.
14. Chevrier C, Dananché B, Bahuau M, et al. Occupational exposure to organic solvent mixtures during pregnancy and the risk of non-syndromic oral clefts. Occupational and Environmental Medicine. 2006;63(9):617-623.
15. Cordier S, Garlantezec R, Labat L, et al. Exposure during pregnancy to glycol ethers and chlorinated solvents and the risk of congenital malformations. Epidemiology. 2012;23(6):806-812.
16. Laumon B, Martin JL, Collet P, Bertucat I, Verney MP, Robert E. Exposure to organic solvents during pregnancy and oral clefts: A case-control study. Reprod Toxicol. 1996;10(1):15-19.

17. Holmberg PC, Kurppa K. An epidemiologic approach to the study of possible teratogenic effects of chemical and physical environments. Scand J Work Environ Health. 1982;8(Suppl. 1):89-91.

18. Shaw GM, Nelson V, Iovannisci DM, Finnell RH, Lammer EJ. Maternal occupational chemical exposures and biotransformation genotypes as risk factors for selected congenital anomalies. Am J Epidemiol. 2003;157(6):475-484.

19. Romitti PA, Herring AM, Dennis LK, Wong-Gibbons DL. Meta-analysis: Pesticides and orofacial clefts. Cleft Palate Craniofac J. 2007;44(4):358-365.

20. Yang W, Carmichael SL, Roberts EM, et al. Residential agricultural pesticide exposures and risk of neural tube defects and orofacial clefts among offspring in the san joaquin valley of california. Am J Epidemiol. 2014;179(6):740-748.

21. Hao Y, Tian S, Jiao X, et al. Association of parental environmental exposures and supplementation intake with risk of nonsyndromic orofacial clefts: A case-control study in heilongjiang province, china. Nutrients. 2015;7(9):7172-7184.

22. Greenlees R, Neville A, Addor MC, et al. Paper 6: EUROCAT member registries: Organization and activities. Birth Defects Res A Clin Mol Teratol. 2011;91 Suppl 1:S5-S100.

23. Matheson MC, Benke G, Raven J, et al. Biological dust exposure in the workplace is a risk factor for chronic obstructive pulmonary disease. Thorax. 2005;60(8):645-651.

24. Kromhout H, Vermeulen R. Application of job-exposure matrices in studies of the general population: Some clues to their performance. Eur Respir Rev. 2001(11):80-90.

25. International Labor Office. International standard classification of occupations. 1988.

26. Health Council of the Netherlands. Towards an optimal use of folic acid. 2008;2008/02E(06/01).

27. Nurminen T, Rantala K, Kurppa K, Holmberg PC. Agricultural work during pregnancy and selected structural malformations in finland. Epidemiology. 1995;6(1):23-30.

28. 't Mannetje A, Kromhout H. The use of occupation and industry classifications in general population studies. Int J Epidemiol. 2003;32(3):419-428.

29. Teschke K, Abanto Z, Arbour L, et al. Exposure to anesthetic gases and congenital anomalies in offspring of female registered nurses. Am J Ind Med. 2011;54(2):118-127.

30. Bolognesi C. Genotoxicity of pesticides: A review of human biomonitoring studies. Mutat Res. 2003;543(3):251-272.

31. Brender JD, Zhan FB, Langlois PH, Suarez L, Scheuerle A. Residential proximity to waste sites and industrial facilities and chromosomal anomalies in offspring. Int J Hyg Environ Health. 2008;211(1-2):50-58.

CHAPTER 4

Maternal occupational exposure to endocrine disrupting chemicals and urogenital anomalies in the offspring

Nynke Spinder
Jorieke E.H. Bergman
Martie van Tongeren
H. Marike Boezen
Hans Kromhout
Hermien E.K. de Walle

Submitted

ABSTRACT

Background Urogenital anomalies comprise any defect of the organs and tissues responsible for the formation and excretion of urine. The aetiology of urogenital anomalies is largely unknown.

Objective To examine the association between maternal occupational exposure to endocrine disrupting chemicals (EDCs) and subgroups of urinary anomalies and hypospadias in offspring.

Methods For this case–control study we selected cases with urogenital anomalies from Eurocat Northern Netherlands and non-malformed controls from the Lifelines children cohort born between 1997 and 2013. Information on maternal jobs held early in pregnancy was collected via self-administered questionnaires. Job titles were translated into occupational exposure to EDCs using a job exposure matrix. Adjusted odds ratios (aORs) and 95% confidence intervals (95%CIs) were estimated to assess the association between maternal occupational exposure to EDCs and to specific types of EDCs and urinary anomalies and hypospadias.

Results This study included 530 cases with urogenital anomalies, 364 cases with hypospadias, and 5602 non-malformed controls. For urinary anomalies and hypospadias, 23.1% and 22.9% of the cases were exposed to EDCs respectively, whereas 19.8% of the controls were exposed. We found an association between maternal occupational exposure to organic solvents/ alkylphenolic compounds and urinary anomalies (aOR 1.41, 95%CI 1.01,1.97) that became stronger when combinations of urinary anomalies co-occurred with other defects (aOR 7.51, 95%CI 2.41,23.43). An association was also observed for exposure to phthalates/benzophenones/parabens/siloxanes and urinary anomalies (aOR 1.56, 95%CI 1.06, 2.29), specifically urinary collecting system anomalies (aOR 1.62, 95% CI 1.03, 2.54) and combinations of urinary anomalies (aOR 2.90, 95%CI 1.09, 7.71). We observed no association between EDC exposure and hypospadias.

Conclusion Maternal occupational exposure to specific EDCs can increase the risk of urinary anomalies in offspring, and this should be taken into consideration when carrying out risk assessments of the workplace.

INTRODUCTION

Urogenital anomalies are congenital anomalies representing any defect in the organs and tissues responsible for the formation and excretion of urine. The total prevalence of kidney and urinary collecting system anomalies is 31 per 10,000 births in Europe [1].These anomalies comprise a broad range of disorders that result from abnormal embryonal renal development, such as renal parenchymal malformations, abnormalities in renal migration, or abnormalities of the urinary collecting system. The severity of urinary anomalies can differ. Total absence of the kidneys will cause neonatal death, whereas milder kidney and urinary collecting system anomalies (e.g. vesico-ureteral-renal reflux – the retrograde passage of urine from the bladder into the upper urinary tract) can lead to chronic renal failure if untreated [2].

Hypospadias is the most common genital anomaly, with a total prevalence of 14 per 10,000 births in Europe [1]. Hypospadias is present only in males and is characterised by an abnormal position of the urethral opening that ranges from the urethral opening being near the tip of the penis to further down the shaft of the penis, scrotum or in the perineum. Most types of hypospadias need surgical correction after birth.

Foetal development occurs under influence of hormones. It has been proposed that hypospadias develops through disruption of the androgenic stimulation required for the development of the normal male external genitalia. Both genetic and environmental factors can negatively affect androgenic stimulation [3]. For urinary anomalies the pathogenesis is largely unknown, but both genetic and environmental factors are thought to be involved [4]. Exposure to certain chemicals can influence hormonal activity and adversely affect foetal development of the urogenital tract [5]. These potentially endocrine disrupting chemicals (EDCs) can be man-made or naturally occurring. Sources of EDC exposure in the general population are diet, personal care products, cosmetics, plastics, textiles, and construction materials [6,7]. However, a relatively high level of exposure may occur in specific occupations as EDCs are present in a large variety of materials and products used in the workplace, such as pesticides, phthalates, and organic solvents, or can be by-products formed during manufacturing, for example dioxins [6,7].

Several studies have found an association between maternal occupational exposures to EDCs and hypospadias [8,9], whilst a few other studies have found associations between specific EDCs, such as pesticides [10] and solvents [11,12], and hypospadias. However, other

studies have found no association between maternal occupational exposure to pesticides or metals and increased risk of hypospadias [13,14].

Studies regarding maternal occupational exposure early in pregnancy in relation to urinary anomalies are limited. One study found an association between maternal occupational exposure to solvents and urinary malformations [15]. However, only 13 cases were included in this prospective study. Another study found no association between solvent exposure and urinary malformations [16]. Neither of these studies differentiated between specific subcategories of urinary anomalies. The aim of our study was thus to examine the association between maternal occupational exposure to EDCs early in pregnancy and subgroups of urinary anomalies and hypospadias in the offspring.

METHODS

Study design

This is a case–control study. Cases were selected from European Concerted Action on Congenital Anomalies and Twins Northern Netherlands (Eurocat NNL). Since 1981 this registry has been collecting data on children born with congenital anomalies in the northern Dutch provinces of Groningen, Friesland, and Drenthe. Eurocat NNL registers live births (up to 10 years of age at notification), stillbirths, miscarriages, and pregnancies terminated because of a congenital anomaly. After the parents have given informed consent, they are asked to complete a questionnaire that includes items on pregnancy characteristics, parental medical history, demographic and occupational characteristics, pre-pregnancy weight and height, and smoking, alcohol, and medication use during the periconceptional period (3 months before pregnancy through the end of the first trimester).

Controls for this study were selected from the Lifelines cohort. Lifelines is a multi-disciplinary prospective population-based cohort study examining in a three-generation design the health and health-related behaviours of 167,729 persons living same catchment region as Eurocat NNL. Participants were recruited through general practitioners in the study area. Children were invited to participate if one parent was already a participant in Lifelines. Children were included from 6 months of age until their 18th birthday after parents or the child has given informed consent. Parents of participating children received a questionnaire with questions on pregnancy, childbirth, and health of the child in the first

6 months of life. Detailed information about the Lifelines cohort study has been published previously [17].

Case and control definition

Children, foetuses, and terminated pregnancies affected by a major anomaly of the urogenital tract born between 1997 until 2013 were selected from Eurocat NNL. Coding and classification of congenital anomalies was performed according to EUROCAT guidelines [18,19]. Since there are many different types of urinary tract anomalies, we classified cases into four groups of urinary anomalies: (I) malformations of the renal parenchyma, (II) anomalies of the urinary collecting system, (III) abnormal embryonic migration of kidneys and other urinary tract anomalies, and (IV) combinations of urinary anomalies [4,20]. The anomalies included in these categories are listed in Table 1. The primary urinary tract anomaly was used for categorisation. For example, if a child developed renal dysplasia as a consequence of an ureteropelvic junction stenosis (UPJ stenosis), the anomaly was classified as UPJ stenosis and categorized under "anomalies of the urinary collecting system". The only genital tract anomaly included in this study was hypospadias (Table 1). Infants with both hypospadias and urinary anomalies were counted in both main categories (n=7). Cases with a genetic or chromosomal anomaly were excluded. To avoid genetic correlation, we also excluded cases in which a sibling with the same defect was included.

Controls were defined as children without congenital anomalies born between 1997 and 2013. We only selected infants whose biological mother participated in Lifelines. Parents were asked if their child was born with a congenital anomaly. Since linkage with Eurocat NNL was not possible and parental descriptions of the congenital anomaly were poor, we could not include these infants as cases. One infant per mother was included to avoid genetic correlation.

Table 1 | Classes of urogenital anomalies

Anomaly	Classes	Included urinary tract anomalies
Urinary anomalies	I Malformations of the renal parenchyma	Renal agenesis, renal hypoplasia, multicystic dysplastic kidneys, cystic kidney, and renal dysplasia
	II Anomalies of the urinary collecting system	Hypdronephrosis (end stage of obstructive anomalies), ureteropelvic junction stenosis, megaloureter, hydroureter, duplication of ureter, vesico-uretero-renal reflux, epispadias, exstrophy of urinary bladder, OEIS complex, (posterior) urethral valves, stenosis, atresia of urethra and bladder neck, and absence of bladder and urethra
	III Abnormal embryonic migration of kidneys and other urinary tract anomalies	Pelvic kidney, horseshoe kidney, other malformations of the urinary system
	IV Combinations of urinary anomalies	Presence of at least two types of urinary tract anomalies, both considered to be primary anomalies and belonging to at least two categories
Genital anomalies	Hypospadias	Glandular*, coronal, penile, penoscrotal and perineal hypospadias

*Included from birth year 2005 onwards according to EUROCAT guidelines [17,18]

Exposure assessment

Mothers were asked to report the job, including industry of employment, they held during early pregnancy. The maternal job descriptions of children included from the Eurocat registry and Lifelines cohort were coded by two authors (NS, HK) into the International Standard Classification of Occupational 1988 (ISCO88) [21], without knowledge of case or study details. To translate the ISCO88 codes into occupational exposure, we used a Job Exposure Matrix (JEM) developed by van Tongeren and colleagues. The original JEM was developed to study the risk of hypospadias after maternal exposure to EDCs [22] and later updated to improve its performance [6]. Chemicals incorporated in this JEM were identified from literature and classified into nine chemical groups: polycyclic aromatic hydrocarbons (PAHs), polychlorinated organic compounds, pesticides, phthalates, organic solvents, bisphenol A, alkylphenolic compounds, brominated flame retardants, and a miscellaneous group (benzophenones, parabens, and siloxanes). Three occupational experts scored the exposure to each chemical group as "unlikely, possible or probable" using job titles form the Standard Occupation Classification (SOC). Two authors (NS, HK) translated SOC codes into the ISCO88 classification to make the JEM applicable to the data in the present study. Due to sparse data (<5 exposed cases) exposures to polychloride organic compounds, bisphenol A, and flame retardants were not analysed. Detailed information on the JEM has been published elsewhere [6].

Statistical analyses

Infant and maternal characteristics for cases and controls were tabulated. Variables registered in both Eurocat NNL and the Lifelines cohort were: child sex, birth year, maternal age at delivery, maternal body mass index (BMI) – the self-reported pre-pregnancy weight and height for Eurocat NNL cases or objective measurement at baseline visit for Lifelines controls (underweight [<18.5 kg/m^2], normal [18.5-24.9 kg/m^2], overweight [25-29.9 kg/m^2], or obese [≥30 kg/m^2] –, maternal education level (low [primary school, lower vocational education, pre-vocational education], middle [secondary vocational education, general secondary education or pre-university education], or high [higher professional education or academic education]), maternal smoking (no, yes/some period during pregnancy), maternal alcohol use during pregnancy (no, yes/some period during pregnancy), folic acid use (no/wrong period, yes/sometime during periconceptional period), and fertility problems (no, yes [self-reported fertility problems or fertility treatment]).

The correlation between EDC subgroups was explored in mothers of controls to determine the best modelling strategy. Due to high correlation, organic solvents and alkylphenolic compounds were combined into a single exposure group (exposure to at least one exposure in this group), as were phthalates, benzophenones, parabens, and siloxanes.

The association between maternal occupational exposures was assessed separately for the four classes of urinary anomalies and hypospadias. Because hypospadias is only present in boys, only boys were selected as controls for the hypospadias analyses. The association between any occupational exposures (possible/probable) and urinary anomalies and hypospadias were assessed using univariate and multivariate logistic regression to estimate crude odds ratios (OR) and adjusted odds ratios (aOR), respectively. Cases and controls with no occupational exposure to EDCs included in the analysis were used as reference category. Multivariate logistic regression analyses were adjusted for birth year, maternal age at delivery, maternal BMI, and smoking and alcohol use during pregnancy, based on chi-square tests (Supplementary Table 1). Multivariate logistic regressions regarding urinary anomalies were additionally adjusted for child sex, folic acid use, and fertility problems. Stratified analyses were conducted for isolated urogenital defects and for urogenital defects co-occurring with congenital anomalies because these may differ in aetiology. To account for the likelihood of exposure, subgroup analyses were performed only including mothers who had a job 'probably' exposed to EDCs, meaning that exposure was likely to occur in more than 10% of workers with this job. We also performed analyses using 'no

exposure' to any occupational EDCs as the reference category. The Statistical Package for the Social Sciences version 23 (SPSS v23) was used to perform all analysis.

RESULTS

We selected 1,462 cases with urogenital defects from Eurocat NNL (Figure I). Cases were excluded because mothers had no job early in pregnancy (n=176) or because job information was missing (n=369). Fourteen cases were excluded because siblings with the same defect were included. We included 530 cases with urinary anomalies, 364 cases with hypospadias, and 7 cases with both a urinary anomaly and hypospadias.

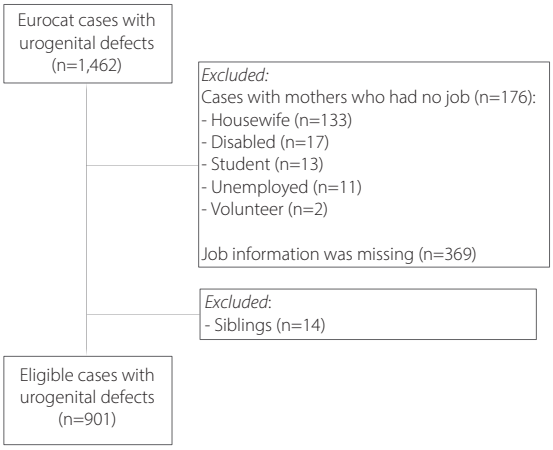


Figure 1 | Flowchart case selection from Eurocat Northern Netherlands, 1997-2013

The Lifelines children's cohort consisted of 12,494 potentially eligible infants born from 1997 to 2013 (Figure II). From these, we excluded 163 non-biological infants. A further 814 children were excluded because their parents reported one or more congenital anomalies or it was unknown if the child was born with a congenital anomaly. Another 3,029 children were excluded because their mother did not work early in pregnancy or job information was missing. One child per parent was selected, which resulted in exclusion of another 2,886 children. In total, 5,602 children without congenital anomalies were selected as control group.

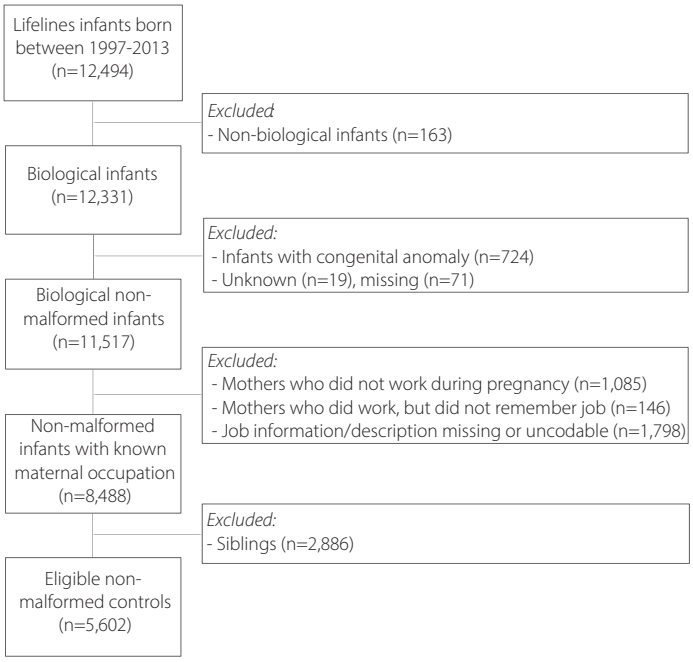


Figure 2 | Flowchart control selection from Lifelines population based cohort study Northern Netherlands, 1997-2013

Children affected by a urinary anomaly were more often boys (70.0% of Eurocat NNL cases were male versus 48.8% of the Lifelines cohort, Supplementary Table 1). For both urinary anomalies and hypospadias cases, the mothers were younger at delivery and had a lower BMI compared to controls. Mothers of cases were also more often smokers and more often used alcohol during pregnancy compared to controls. The mothers of urinary anomaly cases used folic acid less often and more often had fertility problems.

Urinary anomalies

The percentage of women exposed to EDCs and ORs for urinary anomalies are shown in Table 2. For urinary anomalies, 23.1% of the cases and 19.8% of the controls were exposed to 'any' EDCs. After adjustment, an OR of 1.21 (95%CI 0.96-1.53) was observed between exposure to 'any' EDCs and urinary anomalies when comparing to non-exposed infants. When we looked into specific types of EDCs, we observed associations for exposure to organic solvents/alkylphenolic compounds (aOR 1.41, 95%CI 1.01-1.97) and exposure to phthalates/benzophenones/parabens/siloxanes (aOR 1.56, 95%CI 1.06-2.29). No associations

were observed for exposure to PAHs or pesticides when comparing to non-exposed infants (aOR 1.06, 95%CI 0.73-1.53 and aOR 0.53, 95%CI 0.22-1.25 respectively).

Subgroup analyses for specific classes of urinary anomalies were performed. The aOR for anomalies of the urinary collecting system was higher compared to all urinary anomalies (aOR 1.29, 95%CI 0.98-1.69). The aORs for other subgroups anomalies were lower and ranged from 0.84 (95%CI 0.28-2.52) for abnormal embryonic migration of the kidneys to 1.10 (95%CI 0.51-2.35) for combinations of urinary tract anomalies. An association was found between occupational exposure to phthalates/benzophenones/parabens/siloxanes and anomalies of the urinary collecting system (aOR 1.62, 95%CI 1.03-2.54) and combinations of urinary anomalies (aOR 2.90, 95%CI 1.09-7.71). We found no associations with anomalies of the renal parenchyma and abnormal embryonic migration of the kidney.

When we performed a stratified analysis for isolated urinary anomalies (n=420, Supplementary Table 2) and urinary anomalies co-occurring with other congenital anomalies (n=117, Supplementary Table 3), the association between occupational exposure to phthalates/benzophenones/parabens/siloxanes was stronger for isolated cases (aOR 1.63, 95%CI 1.07-2.49) than for urinary defects co-occurring with congenital anomalies (aOR 1.20, 95%CI 0.51-2.80), specifically for anomalies of the urinary collecting system (aOR 1.76, 95%CI 1.11-2.79). The association between exposure to organic solvents/alkylphenolic compounds and combinations of urinary anomalies became stronger when restricting the analysis to urinary defects co-occurring with congenital anomalies (aOR 7.51, 95%CI 2.41-23.43).

Subgroup analyses performed for mothers with 'probable' exposure to EDCs according to the JEM and for women not exposed to any EDC (the reference category) did not materially change the results (Supplementary Table 4 and 5).

Table 2 | Prevalence, crude OR and adjusted OR of maternal occupational exposure to EDCs and the risk of urinary anomalies in the offspring (Eurocat) compared to non-malformed controls (Lifelines), North Netherlands, 1997-2013

	Total	Unexposed		Exposed		Unadjusted		Adjusted[a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Any EDC									
Controls	5602	4491	(80.2%)	1111	(19.8%)	1.00		1.00	
Urinary anomalies	537	413	(76.9%)	124	(23.1%)	1.21	(0.98-1.50)	1.21	(0.96-1.53)
Malformations of the renal parenchyma	109	85	(78.0%)	24	(22.0%)	1.14	(0.72-1.80)	1.04	(0.64-1.69)
Anomalies of the urinary collecting system	360	274	(76.1%)	86	(23.9%)	1.27	(0.99-1.63)	1.29	(0.98-1.69)
Abnormal embryonic migration of kidneys	25	20	(80.0%)	5	(20.0%)	1.01	(0.38-2.67)	0.84	(0.28-2.52)
Combination of urinary tract anomalies	43	34	(79.1%)	9	(20.9%)	1.07	(0.51-2.24)	1.10	(0.51-2.35)
PAHS									
Controls	5602	5251	(93.7%)	351	(6.3%)	1.00		1.00	
Urinary total	537	498	(92.7%)	39	(7.3%)	1.17	(0.83-1.65)	1.06	(0.73-1.53)
Malformations of the renal parenchyma	109	104	(95.4%)	5	(4.6%)	0.72	(0.29-1.78)	0.51	(0.18-1.41)
Anomalies of the urinary collecting system	360	330	(91.7%)	30	(8.3%)	1.36	(0.92-2.01)	1.28	(0.84-1.94)
Abnormal embryonic migration of kidneys	25	<5				NC		NC	
Combination of urinary tract anomalies	43	<5				NC		NC	
Pesticides									
Controls	5602	5486	(97.9%)	116	(2.1%)	1.00		1.00	
Urinary total	537	531	(98.8%)	6	(1.1%)	0.53	(0.23-1.22)	0.53	(0.22-1.25)
Malformations of the renal parenchyma	109	<5				NC		NC	
Anomalies of the urinary collecting system	360	355	(98.6%)	5	(1.4%)	0.67	(0.27-1.64)	0.65	(0.25-1.66)
Abnormal embryonic migration of kidneys	25	<5				NC		NC	
Combination of urinary tract anomalies	43	<5				NC		NC	

Table 2. Continued

	Total	Unexposed		Exposed		Unadjusted		Adjusted[a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Organic Solvents/Alkylphenolic compounds[b]									
Controls	5602	5220	(93.2%)	382	(6.8%)	1.00		1.00	
Urinary total	537	486	(90.5%)	51	(9.5%)	1.43	(1.06-1.95)	1.41	(1.01-1.97)
Malformations of the renal parenchyma	109	100	(91.7%)	9	(8.3%)	1.23	(0.62-2.45)	1.17	(0.58-2.38)
Anomalies of the urinary collecting system	360	326	(90.6%)	34	(9.4%)	1.43	(0.99-2.06)	1.39	(0.93-2.06)
Abnormal embryonic migration of kidneys	25	<5				NC		NC	
Combination of urinary tract anomalies	43	37	(86.0%)	6	(14%)	2.22	(0.93-5.28)	2.16	(0.88-5.30)
Phthalates/Benzophenones/Parabens/Siloxanes[b]									
Controls	5602	5348	(95.5%)	254	(4.5%)	1.00		1.00	
Urinary total	537	500	(93.1%)	37	(6.9%)	1.56	(1.09-2.23)	1.56	(1.06-2.29)
Malformations of the renal parenchyma	109	104	(95.4%)	5	(4.6%)	1.01	(0.41-2.51)	0.92	(0.36-2.32)
Anomalies of the urinary collecting system	360	334	(92.8%)	26	(7.2%)	1.64	(1.08-2.49)	1.62	(1.03-2.54)
Abnormal embryonic migration of kidneys	25	<5				NC		NC	
Combination of urinary tract anomalies	43	38	(88.4%)	5	(11.6%)	2.77	(1.08-7.10)	2.90	(1.09-7.71)

[a] adjusted for child sex, birth year, maternal age and body mass index, smoking, alcohol, and folic acid use during pregnancy, and fertility problems. [b] exposure to at least one exposure in this group. EDCs = endocrine disrupting chemicals, PAH = polycyclic aromatic hydrocarbons, NC = not calculated due to sparse data (<5 exposed cases).

Hypospadias

The exposure rates and ORs for hypospadias are shown in Table 3. For hypospadias, 22.9% of the cases and 18.9% of the controls were exposed to 'any' EDCs. We observed no association between EDCs in general and hypospadias in offspring (aOR 1.26, 95%CI 0.95-1.65), nor did we observe an association between subcategories of EDCs and hypospadias.

We performed stratified analyses of isolated hypospadias (n=341) and hypospadias co-occurring with other congenital anomalies (n=30) (Supplementary Table 6). An increased aOR for 'any' EDCs exposure and hypospadias that co-occurred with another congenital anomalies (aOR 1.46, 95%CI 0.63-3.39) as compared to isolated hypospadias (aOR 1.23, 95%CI 0.92-1.64). Subgroup analyses for mothers with 'probable' exposure to EDCs or using women not exposed to any EDC as reference category did not materially change the results (Supplementary Table 7 and 8).

Table 3 | Prevalence, crude OR, and adjusted OR of maternal occupational exposure to EDCs and the risk of hypospadias in the offspring (Eurocat) compared to non-malformed controls (Lifelines), North Netherlands, 1997-2013

	Total	Unexposed		Exposed		Unadjusted		Adjusted [a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Any EDC									
Controls [b]	2731	2214	(81.1%)	517	(18.9%)	1.00		1.00	
Hypospadias	371	286	(77.1%)	85	(22.9%)	1.27	(0.98-1.65)	1.26	(0.95-1.65)
PAHS									
Controls [b]	2731	2560	(93.7%)	171	(6.3%)	1.00		1.00	
Hypospadias	371	340	(91.6%)	31	(8.4%)	1.37	(0.92-2.03)	1.37	(0.91-2.07)
Pesticides									
Controls [b]	2731	2685	(98.3%)	46	(1.7%)	1.00		1.00	
Hypospadias	371	366	(98.7%)	5	(1.3%)	0.80	(0.32-2.02)	0.76	(0.30-1.98)
Organic Solvents/ Alkylphenolic compounds[c]									
Controls [b]	2731	2641	(96.7%)	90	(3.3%)	1.00		1.00	
Hypospadias	371	347	(93.5%)	24	(6.5%)	1.02	(0.66-1.59)	0.94	(0.59-1.48)
Phthalates/Benzophenones/ Parabens/Siloxanes[c]									
Controls [b]	2731	2620	(95.9%)	11	(4.1%)	1.00		1.00	
Hypospadias	371	351	(95.6%)	20	(5.4%)	1.35	(0.83-2.19)	1.21	(0.73-2.01)

[a] adjusted for birth year, maternal age and body mass index, smoking and alcohol use during pregnancy, [b] only boys are selected as controls. [c] exposure to at least one exposure in this group. EDCs = endocrine disrupting chemicals, PAH = polycyclic aromatic hydrocarbons.

DISCUSSION

Principal findings

In this study we found an association between maternal occupational exposure to organic solvents/alkylphenolic compounds and phthalates/benzophenones/parabens/siloxanes and urinary anomalies, specifically for anomalies of the urinary collecting system and when more than one urinary anomaly was present. Women exposed to organic solvents/alkylphenolic compounds were working in the agricultural sector or as life science technicians. Women exposed to phthalates/benzophenones/parabens/siloxanes worked mainly as cleaners, hairdressers or beauticians.

Strengths and weaknesses of the study

For this case–control study we used case data from the high-quality population-based Eurocat NNL registry. Detailed medical information was available for each case, and all cases were coded by trained registry staff according to international coding guidelines [18]. These factors made it possible to distinguish between classes of urinary anomalies and between isolated and urinary defects that co-occurred with other anomalies. A major strength of this study is that we used non-malformed controls. We selected controls from Lifelines, a large population-based cohort which recruited its participants from the same region as Eurocat. The Lifelines cohort is representative for the population in the Northern Netherlands [23]. Another strength is that we used a JEM for occupational exposure assessment. When compared to self-reported exposures, use of a JEM limits the effect of recall bias on exposure estimates as well as the differential misclassification of exposure compared to self-reported exposure [24,25].

The different study designs of Eurocat and Lifelines could have introduced differential information bias. Eurocat aimed to conduct research specifically to identify risk factors for congenital anomalies, whereas Lifelines aimed to collect data that could be used to examine diseases in general. Eurocat questionnaires therefore focused specifically on risk factors for congenital anomalies, whereas the Lifelines questionnaire included a wide variety of risk factors for neonatal and childhood diseases such as low birth weight, asthma, allergies, and congenital anomalies. This is of particular importance when assessing our confounder variables because questions were asked with different intentions. However, differential bias was unlikely for our main risk factor of interest: maternal occupational exposure early in pregnancy. In both questionnaires, mothers were asked to report their job during pregnancy. In addition, recall bias could have been introduced, as the

time between birth and notification was 16 months for Eurocat cases and 7.5 years for Lifelines controls. The effect of recall bias on occupational exposure is expected to be small, because exposure is assigned based on job description/industry of employment, because exposure is assigned based on job description/industry of employment, and recall bias for self-reported job description has been reported to be limited [26]. A limitation of JEMs in general is that they assign exposure at job level, which under normal conditions will result in a Berkson type error resulting in unbiased, but less precise, risk estimates. Another limitation is that the JEM estimates exposure as possible exposed (involves less than 10% of workers with this job title) and probably exposed (exposure was likely to occur in more than 10% of workers with this job) [6]. No studies have evaluated how the exposure assigned by the JEM relates to the actual exposures of the females involved. We also have no information about the number of hours mothers per week worked early in pregnancy or other characteristics regarding employment. Therefore, it is possible that the actual exposure is lower or absent than the exposure estimated by the JEM. One other limitation is that the JEM assigns a probability of exposure rather than a level of exposure, which precludes performing dose–response analyses.

Interpretation

Studies regarding occupational exposure and urinary anomalies are scarce and only performed for maternal occupational exposure to solvents. One earlier study also found an association between solvent exposure and urinary anomalies [15], but the results of two other studies were not in line with our finding [16,27]. However, one of these studies did find an association between glycol ethers (a specific group of organic solvents) and multiple congenital anomalies [16], which is in line with the association we found in our subgroup analysis for cases with a combination of urinary anomalies defects co-occurring with congenital anomalies. We did not observe a specific pattern of co-occurring congenital anomalies in the five exposed cases that could explain the association between solvents and multiple anomalies. The number of included urinary cases in those previous studies was low compared to the present study (n=12-76 versus n=537), and therefore no subgroup analyses were performed.

It is known that EDCs can interfere with hormone levels during foetal development, and hormones such as gonadotropins, oestrogen, and androgens play an important role in development of many tissues during foetal development. Therefore, it seems possible that maternal occupational exposure to solvents increases the risk of multiple

co-occurring congenital anomalies. Additionally, it has been suggested that exposure to EDCs is associated with inappropriate modulation of hormone receptors and can therefore interfere with development of the (male) reproductive tract [28]. The development of the urinary system is closely related to the development of the reproductive tract.

However, despite the hypothesis that EDCs can interfere with development of the reproductive tract, we did not find an association between maternal occupational exposure to EDCs and hypospadias, nor did we find one with specific groups of EDCs. Previous studies have reported contradictory results. Four studies found an association between maternal occupational exposure to EDCs in general and hypospadias [8,29-31], but several other studies and our study found no association [32-35]. A few studies performed subgroup analysis for specific EDCs [29,30,32-35]. One study that found an effect for EDC exposure in general, found that specifically phthalates and other compounds (e.g. parabens) had an effect [29]. However, another study that found an association, did not find an effect for specific EDC subgroups [30]. Three studies that found no association between EDC exposure and hypospadias, found also no specific subgroup effects [32,34,35]. Only one study found an association between maternal occupational exposure to metals and mild hypospadias [33]. Two studies showing an association between EDC exposure and hypospadias included a small number of cases (n=57-80), which could have resulted in imprecise estimates [8,30]. As studies were conducted in different countries, it is possible that exposure levels varied over time and by country due to (pregnancy) policy differences. The difference in results could not be explained by exposure assessment methods as all studies used the same JEM by van Tongeren and colleagues [22].

Conclusion

This study showed an association between maternal occupational exposure to organic solvents/alkylphenolic compounds and phthalates/benzophenones/parabens/siloxanes early in pregnancy, and urinary anomalies in offspring. We also found some signals that exposure to those specific EDCs is associated with a combination of urinary anomalies that co-occur with other congenital anomalies. In the Netherlands, employers are obliged by law to identify occupational risks for pregnant employees by carrying out risk assessments at the workplace since 1997 [36]. Women, their healthcare providers, and their employers need to be aware that occupational exposure to specific EDCs early in pregnancy may be associated with urinary anomalies in offspring. Occupational hygienist should be consulted to take exposure to those specific EDCs into consideration when risk assessments are carried out at the workplace.

REFERENCES

1. EUROCAT. Prevalence charts and tables. https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-data/prevalence. Updated 2017. Accessed 1/15, 2020.
2. Salmasi G, Grady R, Jones J, McDonald SD, Knowledge Synthesis Group. Environmental tobacco smoke exposure and perinatal outcomes: A systematic review and meta-analyses. Acta Obstet Gynecol Scand. 2010;89(4):423-441.
3. Laurence S Baskin. Hypospadias: Pathogenesis, diagnosis, and evaluation. https://www.uptodate.com/contents/hypospadias-pathogenesis-diagnosis-and-evaluation?search=hypospadias&source=search_result&selectedTitle=1~75&usage_type=default&display_rank=1. Updated 2019. Accessed Oct 23, 2019.
4. Rosenblum ND. Overview of congenital anomalies of the kidney and urinary tract (CAKUT). https://www.uptodate.com/contents/overview-of-congenital-anomalies-of-the-kidney-and-urinary-tract-cakut?source#H12. Updated 2017. Accessed 9/23, 2019.
5. Yiee JH, Baskin LS. Environmental factors in genitourinary development. J Urol. 2010;184(1):34-41.
6. Brouwers MM, van Tongeren M, Hirst AA, Bretveld RW, Roeleveld N. Occupational exposure to potential endocrine disruptors: Further development of a job exposure matrix. Occup Environ Med. 2009;66(9):607-614.
7. World Health Organisation (WHO), United Nations Environment Program, (UNEP). State of the sciense of endocriene disrupting chemicals. 2012:1/296.
8. Haraux E, Braun K, Buisson P, et al. Maternal exposure to domestic hair cosmetics and occupational endocrine disruptors is associated with a higher risk of hypospadias in the offspring. Int J Environ Res Public Health. 2016;14(1):10.3390/ijerph14010027.
9. Ormond G, Nieuwenhuijsen MJ, Nelson P, et al. Endocrine disruptors in the workplace, hair spray, folate supplementation, and risk of hypospadias: Case-control study. Environ Health Perspect. 2009;117(2):303-307.
10. Rocheleau CM, Romitti PA, Dennis LK. Pesticides and hypospadias: A meta-analysis. J Pediatr Urol. 2009;5(1):17-24.
11. Kalfa N, Philibert P, Broussous S, et al. Isolated hypospadias and exposure to endocrine disrupting chemicals during pregnancy: A multi-institutional controlled study in a high prevalence area. Horm Res Paediatr. 2014;82:107.
12. Warembourg C, Botton J, Lelong N, et al. Prenatal exposure to glycol ethers and cryptorchidism and hypospadias: A nested case-control study. Occup Environ Med. 2018;75(1):59-65.
13. Spinder N, Prins JR, Bergman JEH, et al. Congenital anomalies in the offspring of occupationally exposed mothers: A systematic review and meta-analysis of studies using expert assessment for occupational exposures. Hum Reprod. 2019;34:903-919
14. Zhu JL, Hjollund NH, Andersen A-N, Olsen J. Occupational exposure to pesticides and pregnancy outcomes in gardeners and farmers: A study within the Danish national birth cohort. J Occup Environ Med. 2006;48(4):347-352.
15. Garlantézec R, Monfort C, Rouget F, Cordier S. Maternal occupational exposure to solvents and congenital malformations: A prospective study in the general population. Occup Environ Med. 2009;66(7):456-463.

16. Cordier S, Bergeret A, Goujard J, et al. Congenital malformations and maternal occupational exposure to glycol ethers. Epidemiology. 1997;8(4):355-363.

17. Scholtens S, Smidt N, Swertz MA, et al. Cohort profile: LifeLines, a three-generation cohort study and biobank. Int J Epidemiol. 2015;44(4):1172-1180.

18. EUROCAT. EUROCAT guide 1.4: Instruction for the registration of congenital anomalies. 2013.

19. Greenlees R, Neville A, Addor MC, et al. Paper 6: EUROCAT member registries: Organization and activities. Birth Defects Res A Clin Mol Teratol. 2011;91 Suppl 1:S51-S100.

20. Bakker MK, Bergman JEH, Fleurke-Rozema H, et al. Prenatal diagnosis of urinary tract anomalies, a cohort study in the northern netherlands. Prenat Diagn. 2018;38(2):130-134.

21. International Labor Office. International standard classification of occupations. 1988.

22. Van Tongeren M, Nieuwenhuijsen MJ, Gardiner K, et al. A job-exposure matrix for potential endocrine-disrupting chemicals developed for a study into the association between maternal occupational exposure and hypospadias. Ann Occup Hyg. 2002;46(5):465-477.

23. Klijs B, Scholtens S, Mandemakers JJ, Snieder H, Stolk RP, Smidt N. Representativeness of the LifeLines cohort study. PLoS One. 2015;10(9):e0137203.

24. 't Mannetje A, Kromhout H. The use of occupation and industry classifications in general population studies. Int J Epidemiol. 2003;32(3):419-428.

25. Kromhout H, Vermeulen R. Application of job-exposure matrices in studies of the general population: Some clues to their performance. Eur Respir Rev. 2001(11):80-90.

26. Teschke K, Olshan AF, Daniels JL, et al. Occupational exposure assessment in case-control studies: Opportunities for improvement. Occup Environ Med. 2002;59(9):575-93; discussion 594.

27. Cordier S, Szabova E, Fevotte J, Bergeret A, Plackova S, Mandereau L. Congenital malformations and maternal exposure to glycol ethers in the slovak republic. Epidemiology. 2001;12(5):592-593.

28. Yoon K, Kwack SJ, Kim HS, Lee BM. Estrogenic endocrine-disrupting chemicals: Molecular mechanisms of actions on putative human diseases. J Toxicol Environ Health B Crit Rev. 2014;17(3):127-174.

29. Morales-Surez-Varela MM, Toft GV, Jensen MS, et al. Parental occupational exposure to endocrine disrupting chemicals and male genital malformations: A study in the danish national birth cohort study. Environ Health Global Access Sci Sour. 2011;10(1).

30. Giordano F, Abballe A, De Felip E, et al. Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. Birth Defects Res Part A Clin Mol Teratol. 2010;88(4):241-250.

31. Kalfa N, Paris F, Philibert P, et al. Is hypospadias associated with prenatal exposure to endocrine disruptors? A french collaborative controlled study of a cohort of 300 consecutive children without genetic defect. Eur Urol. 2015;68(6):1023-1030.

32. Carbone P, Giordano F, Nori F, et al. The possible role of endocrine disrupting chemicals in the aetiology of cryptorchidism and hypospadias: A population-based case-control study in rural sicily. Int J Androl. 2007;30(1):3-13.

33. Nassar N, Abeywardana P, Barker A, Bower C. Parental occupational exposure to potential endocrine disrupting chemicals and risk of hypospadias in infants. Occup Environ Med. 2010;67(9):585-589.

34. Pierik FH, Burdorf A, Deddens JA, Juttmann RE, Weber RFA. Maternal and paternal risk factors for cryptorchidism and hypospadias: A case-control study in newborn boys. Environ Health Perspect. 2004;112(15):1570-1576.

35. Vrijheid M, Armstrong B, Dolk H, Van Tongeren M, Botting B. Risk of hypospadias in relation to maternal occupational exposure to potential endocrine disrupting chemicals. Occup Environ Med. 2003;60(8):543-550.

36. Burgerlijk Wetboek. Arbeidsomstandighedenbesluit - afdeling 9. zwangere werknemers en werknemers tijdens de lactatie. https://wetten.overheid.nl/jci1.3:c:BWBR0008498&hoofdstuk=1&afdeling=9&artikel=1.41&z=2020-02-01&g=2020-02-01. Updated 1997. Accessed 3/12, 2020.

SUPPLEMENTARY MATERIAL

Supplementary Table 1 | Baseline characteristics of Lifelines controls and Eurocat cases, North Netherlands. 1997-2013

	Controls		Urinary anomalies		Hypospadias	
Occupational exposure	n	(%)	n	(%)	n	(%)
N (total)	5602		537		373	
Child biological sex[e]						
Boy	2731	(48.8%)	377	(70.2%)	373	(100%)
Girl	2871	(51.2%)	160	(29.8%)	0	(0%)
Birth year[e,f]						
1997-2000	1240	(22.1%)	103	(19.2%)	76	(20.4%)
2001-2004	1660	(29.6%)	108	(20.1%)	86	(23.1%)
2005-2008	1293	(23.1%)	131	(24.4%)	115	(30.8%)
2009-2013	1409	(25.2%)	195	(36.3%)	96	(25.7%)
Maternal age at delivery[e,f]						
15-19[a]	3	(0.1%)	1	(0.2%)	1	(0.3%)
20-24	191	(3.6%)	40	(7.5%)	28	(7.5%)
25-29	1492	(28.2%)	167	(31.5%)	128	(34.5%)
30-34	2470	(46.7%)	225	(42.4%)	155	(41.8%)
35-39	1058	(20.0%)	90	(16.9%)	57	(15.4%)
>40	73	(1.4%)	8	(1.5%)	2	(0.5%)
Unknown	315		6		2	
BMI (kg/m^2)[b,e,f]						
Underweight (<18.5)	56	(1.0%)	10	(1.9%)	4	(1.1%)
Normal (18.5-25)	2871	(53.6%)	340	(65.6%)	245	(66.8%)
Overweight (25-30)	1610	(30.1%)	112	(21.6%)	82	(22.3%)
Obese (>30)	818	(15.3%)	56	(10.8%)	36	(9.8%)
Unknown	247		19		6	
Education level						
Low	649	(12.3%)	56	(10.7%)	38	(10.3%)
Middle	2396	(45.4%)	260	(49.7%)	185	(50.3%)
High	2236	(42.3%)	207	(39.6%)	145	(39.4%)
Unknown	321		14		5	
Smoking during pregnancy[c,e,f]						
No	5036	(90.2%)	415	(78.3%)	300	(81.1%)
Yes	549	(9.8%)	115	(21.7%)	70	(18.9%)
Unknown	17		7		3	
Alcohol during pregnancy[c,e,f]						
No	5045	(90.3%)	406	(76.7%)	281	(76.2%)
Yes	544	(9.7%)	123	(23.3%)	88	(23.8%)
Unknown	13		8		4	

Supplementary Table 1. Continued

	Controls		Urinary anomalies		Hypospadias	
Occupational exposure	n	(%)	n	(%)	n	(%)
Folic acid use[e]						
No	847	(16.5%)	139	(26.3%)	71	(19.2%)
Yes	4272	(83.5%)	389	(73.7%)	299	(80.8%)
Unknown	483		9		3	
Fertility problems[e]						
No	5230	(93.9%)	481	(90.9%)	352	(95.1%)
Yes	339	(6.1%)	48	(9.1%)	18	(4.9%)
Unknown	33		8		3	

[a] Lifelines includes participants from 18 years old. [b] BMI of Eurocat cases is based on self-reported weight and length at early pregnancy, whereas weight and length of Lifelines participants is measured at baseline visit at the study clinic. [c] Eurocat reported smoking/alcohol use during pregnancy, whereas Lifelines reported this specifically for the first trimester. [d] only boys were selected as controls for hypospadias. [e] Significant difference between urinary cases and controls (p value <0.05) using X^2 tests. [f] Significant difference between hypospadias cases and controls (p value <0.05) using X^2 tests

Supplementary Table 2 | Prevalence, crude OR, and adjusted OR of maternal occupational exposure to EDCs and the risk of isolated urinary anomalies in the offspring (Eurocat) compared to non-malformed controls (Lifelines), North Netherlands, 1997-2013

	Total	Unexposed		Exposed		Unadjusted		Adjusted[a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Any EDC									
Controls	5602	4491	(80.2%)	1111	(19.8%)	1.00		1.00	
Urinary anomalies	420	326	(77.6%)	94	(22.4%)	1.17	(0.92-1.48)	1.16	(0.90-1.50)
Malformations of the renal parenchyma	64	63	(82.8%)	11	(17.2%)	0.84	(0.44-1.61)	0.78	(0.40-1.54)
Anomalies of the urinary collecting system	315	237	(75.2%)	78	(24.8%)	1.33	(1.02-1.73)	1.32	(0.99-1.76)
Abnormal embryonic migration of kidneys	12	<5				NC		NC	
Combination of urinary tract anomalies	29	<5				NC		NC	
PAHS									
Controls	5602	5251	(93.7%)	351	(6.3%)	1.00		1.00	
Urinary total	420	390	(92.9%)	30	(7.1%)	1.15	(0.78-1.69)	1.06	(0.70-1.61)
Malformations of the renal parenchyma	64	<5				NC		NC	
Anomalies of the urinary collecting system	315	287	(91.1%)	28	(8.9%)	1.46	(0.98-2.18)	1.35	(0.88-2.07)
Abnormal embryonic migration of kidneys	12	<5				NC		NC	
Combination of urinary tract anomalies	30	<5				NC		NC	
Pesticides									
Controls	5602	5486	(97.9%)	116	(2.1%)	1.00		1.00	
Urinary total	420	415	(98.8%)	5	(1.2%)	0.57	(0.23-1.40)	0.56	(0.22-1.43)
Malformations of the renal parenchyma	64	<5				NC		NC	
Anomalies of the urinary collecting system	315	310	(98.4%)	5	(1.6%)	0.76	(0.31-1.88)	0.74	(0.89-1.91)
Abnormal embryonic migration of kidneys	12	<5				NC		NC	
Combination of urinary tract anomalies	29	<5				NC		NC	

Supplementary Table 2. Continued

	Total	Unexposed		Exposed		Unadjusted		Adjusted[a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Organic Solvents/Alkylphenolic compounds[b]									
Controls	5602	5220	(93.2%)	382	(6.8%)	1.00		1.00	
Urinary total	420	382	(91.0%)	38	(9.0%)	1.36	(0.96-1.93)	1.31	(0.90-1.91)
Malformations of the renal parenchyma	64	59	(92.2%)	5	(7.8%)	1.16	(0.46-2.90)	1.09	(0.43-2.80)
Anomalies of the urinary collecting system	315	284	(90.2%)	31	(9.8%)	1.49	(1.02-2.19)	1.42	(0.94-2.15)
Abnormal embryonic migration of kidneys	12	<5				NC		NC	
Combination of urinary tract anomalies	29	<5				NC		NC	
Phthalates/Benzophenones/Parabens/Siloxanes[b]									
Controls	5602	5348	(95.5%)	254	(4.5%)	1.00		1.00	
Urinary total	420	389	(92.6%)	31	(7.4%)	1.68	(1.14-2.47)	1.63	(1.07-2.49)
Malformations of the renal parenchyma	64	<5				NC		NC	
Anomalies of the urinary collecting system	315	290	(92.1%)	25	(7.9%)	1.82	(1.18-2.78)	1.76	(1.11-2.79)
Abnormal embryonic migration of kidneys	12	<5				NC		NC	
Combination of urinary tract anomalies	29	<5				NC		NC	

[a] adjusted for child sex, birth year, maternal age and body mass index, smoking, alcohol, and folic acid use during pregnancy, and fertility problems. [b] exposure to at least one exposure in this group. EDCs = endocrine disrupting chemicals, PAH = polycyclic aromatic hydrocarbons, NC = not calculated due to sparse data (<5 exposed cases).

Supplementary Table 3 | Prevalence, crude OR, and adjusted OR of maternal occupational exposure to EDCs and the risk of urinary anomalies co-occurring with other congenital anomalies in the offspring (Eurocat) compared to non-malformed controls (Lifelines), North Netherlands, 1997-2013

	Total	Unexposed		Exposed		Unadjusted		Adjusted[a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Any EDC									
Controls	5602	4491	(80.2%)	1111	(19.8%)	1.00		1.00	
Urinary anomalies	117	87	(74.4%)	30	(25.6%)	1.39	(0.92-2.12)	1.37	(0.87-2.15)
Malformations of the renal parenchyma	45	32	(71.1%)	13	(28.9%)	1.64	(0.86-3.14)	1.45	(0.72-2.91)
Anomalies of the urinary collecting system	45	37	(82.2%)	8	(17.8%)	0.87	(0.41-1.88)	1.02	(0.46-2.25)
Abnormal embryonic migration of kidneys	13	<5				NC		NC	
Combination of urinary tract anomalies	14	9	(64.3%)	5	(35.7%)	2.25	(0.75-6.71)	2.22	(0.72-6.84)
PAHS									
Controls	5602	5251	(93.7%)	351	(6.3%)	1.00		1.00	
Urinary total	117	108	(92.3%)	9	(7.7%)	1.25	(0.63-2.48)	1.09	(0.52-2.28)
Malformations of the renal parenchyma	45	<5				NC		NC	
Anomalies of the urinary collecting system	45	<5				NC		NC	
Abnormal embryonic migration of kidneys	13	<5				NC		NC	
Combination of urinary tract anomalies	14	<5				NC		NC	
Pesticides									
Controls	5602	5486	(97.9%)	116	(2.1%)	1.00		1.00	
Urinary total	117	<5				NC		NC	
Malformations of the renal parenchyma	45	<5				NC		NC	
Anomalies of the urinary collecting system	45	<5				NC		NC	
Abnormal embryonic migration of kidneys	13	<5				NC		NC	
Combination of urinary tract anomalies	14	<5				NC		NC	

Supplementary Table 3. Continued

	Total	Unexposed		Exposed		Unadjusted		Adjusted[a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Organic Solvents/Alkylphenolic compounds[b]									
Controls	5602	5220	(93.2%)	104	(2.0%)	1.00		1.00	
Urinary total	117	104	(88.9%)	13	(11.1%)	1.71	(0.95-3.07)	1.62	(0.87-3.03)
Malformations of the renal parenchyma	45	<5				NC		NC	
Anomalies of the urinary collecting system	45	<5				NC		NC	
Abnormal embryonic migration of kidneys	13	<5				NC		NC	
Combination of urinary tract anomalies	14	9	(64.3%)	5	(35.7%)	7.59	(0.53-22.76)	7.51	(2.41-23.43)
Phthalates/Benzophenones/Parabens/Siloxanes[b]									
Controls	5602	5348	(95.5%)	254	(4.5%)	1.00		1.00	
Urinary total	117	111	(94.9%)	6	(5.1%)	1.14	(0.50-2.61)	1.20	(0.51-2.80)
Malformations of the renal parenchyma	45	<5				NC		NC	
Anomalies of the urinary collecting system	45	<5				NC		NC	
Abnormal embryonic migration of kidneys	13	<5				NC		NC	
Combination of urinary tract anomalies	14	<5				NC		NC	

[a] adjusted for child sex, birth year, maternal age and body mass index, smoking, alcohol, and folic acid use during pregnancy, and fertility problems. [b] exposure to at least one exposure in this group. EDCs = endocrine disrupting chemicals, PAH = polycyclic aromatic hydrocarbons, NC = not calculated due to sparse data (<5 exposed cases).

Supplementary Table 4 | Prevalence, crude OR and adjusted OR of probable maternal occupational exposure to EDCs and the risk of urinary anomalies in the offspring (Eurocat) compared to non-malformed controls (Lifelines), North Netherlands, 1997-2013.

	Total	Unexposed		Exposed		Unadjusted		Adjusted[a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Any EDC									
Controls	4822	4491	(93.1%)	331	(6.9%)	1.00		1.00	
Urinary anomalies	448	413	(92.2%)	35	(7.8%)	1.15	(0.80-1.65)	0.97	(0.65-1.45)
Malformations of the renal parenchyma	90	85	(94.4%)	5	(5.6%)	0.80	(0.32-1.98)	0.49	(0.17-1.37)
Anomalies of the urinary collecting system	301	274	(91.0%)	27	(9.0%)	1.34	(0.89-2.02)	1.20	(0.77-1.87)
Abnormal embryonic migration of kidneys	21	<5				NC		NC	
Combination of urinary tract anomalies	36	<5				NC		NC	
PAHS									
Controls	5372	5251	(97.7%)	121	(2.3%)	1.00		1.00	
Urinary total	512	498	(97.3%)	14	(2.7%)	1.22	(0.70-2.14)	0.88	(0.47-1.64)
Malformations of the renal parenchyma	106	<5				NC		NC	
Anomalies of the urinary collecting system	340	330	(97.1%)	10	(2.9%)	1.32	(0.68-2.53)	1.08	(0.53-2.19)
Abnormal embryonic migration of kidneys	23	<5				NC		NC	
Combination of urinary tract anomalies	43	<5				NC		NC	
Pesticides									
Controls	5486	98.7	(72.0%)	1.3	(0.2%)	1.00		1.00	
Urinary total	534	<5				NC		NC	
Malformations of the renal parenchyma	496	<5				NC		NC	
Anomalies of the urinary collecting system	358	<5				NC		NC	
Abnormal embryonic migration of kidneys	25	<5				NC		NC	
Combination of urinary tract anomalies	43	<5				NC		NC	

Supplementary Table 4. Continued

	Total	Unexposed		Exposed		Unadjusted		Adjusted[a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Organic Solvents/Alkylphenolic compounds[b]									
Controls	5228	5220	(99.8%)	8	(0.2%)	1.00		1.00	
Urinary total	487	<5				NC		NC	
Malformations of the renal parenchyma	100	<5				NC		NC	
Anomalies of the urinary collecting system	327	<5				NC		NC	
Abnormal embryonic migration of kidneys	23	<5				NC		NC	
Combination of urinary tract anomalies	37	<5				NC		NC	
Phthalates/Benzophenones/Parabens/Siloxanes[b]									
Controls	5522	5348	(96.8%)	174	(3.2%)	1.00		1.00	
Urinary total	531	500	(94.2%)	31	(5.8%)	1.91	(1.29-2.82)	1.91	(1.24-2.93)
Malformations of the renal parenchyma	106	<5				NC		NC	
Anomalies of the urinary collecting system	355	334	(94.1%)	21	(5.9%)	1.93	(1.21-3.08)	1.90	(1.14-3.15)
Abnormal embryonic migration of kidneys	24	<5				NC		NC	
Combination of urinary tract anomalies	39	<5				NC		NC	

[a] adjusted for child sex, birth year, maternal age and body mass index, smoking, alcohol, and folic acid use during pregnancy, and fertility problems. [b] exposure to at least one exposure in this group. EDCs = endocrine disrupting chemicals, PAH = polycyclic aromatic hydrocarbons, NC = not calculated due to sparse data (<5 exposed cases).

Supplementary Table 5 | Prevalence, crude OR, and adjusted OR of maternal occupational exposure to EDCs and the risk of urinary anomalies in the offspring (Eurocat) compared to non-malformed controls and non-exposed as reference category (Lifelines), North Netherlands, 1997-2013

	Total	Unexposed		Exposed		Unadjusted		Adjusted[a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Any EDC									
Controls	5602	4491	(80.2%)	1111	(19.8%)	1.00		1.00	
Urinary anomalies	537	413	(76.9%)	124	(23.1%)	1.21	(0.98-1.50)	1.21	(0.96-1.53)
Malformations of the renal parenchyma	109	85	(78.0%)	24	(22.0%)	1.14	(0.72-1.80)	1.04	(0.64-1.69)
Anomalies of the urinary collecting system	360	274	(76.1%)	86	(23.9%)	1.27	(0.99-1.63)	1.29	(0.98-1.69)
Abnormal embryonic migration of kidneys	25	20	(80.0%)	5	(20.0%)	1.01	(0.38-2.67)	0.84	(0.28-2.52)
Combination of urinary tract anomalies	43	34	(79.1%)	9	(20.9%)	1.07	(0.51-2.24)	1.10	(0.51-2.35)
PAHS									
Controls	4842	4491	(92.8%)	351	(7.2%)	1.00		1.00	
Urinary total	452	413	(91.4%)	39	(8.6%)	1.21	(0.86-1.71)	1.09	(0.75-1.58)
Malformations of the renal parenchyma	90	85	(94.4%)	5	(5.6%)	0.75	(0.30-1.87)	0.50	(0.18-1.41)
Anomalies of the urinary collecting system	304	274	(90.1%)	30	(9.9%)	1.40	(0.95-2.07)	1.12	(0.87-2.00)
Abnormal embryonic migration of kidneys	23	<5				NC		NC	
Combination of urinary tract anomalies	35	<5				NC		NC	
Pesticides									
Controls	4607	4491	(97.5%)	116	(2.5%)	1.00		1.00	
Urinary total	419	416	(98.6%)	6	(1.4%)	0.56	(0.25-1.29)	0.55	(0.23-1.31)
Malformations of the renal parenchyma	86	85	(98.8%)	1	(1.2%)	NC		NC	
Anomalies of the urinary collecting system	279	274	(98.2%)	5	(1.8%)	0.71	(0.29-1.74)	0.68	(0.26-1.76)
Abnormal embryonic migration of kidneys	20	<5				NC		NC	
Combination of urinary tract anomalies	34	<5				NC		NC	

Supplementary Table 5. Continued

	Total	Unexposed		Exposed		Unadjusted		Adjusted[a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Organic Solvents/Alkylphenolic compounds[b]									
Controls	4873	4491	(97.2%)	382	(7.8%)	1.00		1.00	
Urinary total	464	413	(89.0%)	51	(11.0%)	1.45	(1.07-1.98)	1.41	(1.00-1.98)
Malformations of the renal parenchyma	94	85	(90.4%)	9	(9.6%)	1.25	(0.62-2.49)	1.14	(0.56-2.35)
Anomalies of the urinary collecting system	308	274	(89.0%)	34	(11.0%)	1.46	(1.01-2.12)	1.41	(0.95-2.11)
Abnormal embryonic migration of kidneys	22	<5				NC		NC	
Combination of urinary tract anomalies	40	34	(85.0%)	6	(15.0%)	2.08	(0.87-4.97)	1.934	(0.78-4.80)
Phthalates/Benzophenones/Parabens/Siloxanes[b]									
Controls	4745	4491	(94.6%)	254	(5.4%)	1.00		1.00	
Urinary total	450	413	(91.8%)	37	(8.2%)	1.58	(1.11-2.27)	1.55	(1.04-2.29)
Malformations of the renal parenchyma	90	85	(94.4%)	5	(5.6%)	1.04	(0.42-2.59)	0.89	(0.35-2.29)
Anomalies of the urinary collecting system	300	274	(91.3%)	26	(8.7%)	1.68	(1.10-2.56)	1.64	(1.04-2.60)
Abnormal embryonic migration of kidneys	21	<5				NC		NC	
Combination of urinary tract anomalies	39	34	(87.2%)	5	(12.8%)	2.60	(1.01-6.71)	2.55	(0.94-6.88)

[a] adjusted for child sex, birth year, maternal age and body mass index, smoking, alcohol, and folic acid use during pregnancy, and fertility problems. [b] exposure to at least one exposure in this group. EDCs = endocrine disrupting chemicals, PAH = polycyclic aromatic hydrocarbons, NC = not calculated due to sparse data (<5 exposed cases).

Supplementary Table 6 | Prevalence, crude OR, and adjusted OR of maternal occupational exposure to EDCs and the risk of isolated hypospadias and hypospadias co-occurring with other congenital anomalies in the offspring (Eurocat) compared to non-malformed controls and non-exposed as reference category (Lifelines), North Netherlands, 1997-2013

	Total	Unexposed		Exposed		Unadjusted		Adjusted [a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Any EDC									
Controls [b]	2731	2214	(81.1%)	517	(18.9%)	1.00		1.00	
Hypospadias isolated	341	264	(77.4%)	77	(22.6%)	1.25	(0.95-1.64)	1.23	(0.92-1.64)
Hypospadias co-occurring with other CAs	30	22	(73.3%)	8	(26.7%)	1.56	(0.69-3.52)	1.46	(0.63-3.39)
PAHS									
Controls [b]	2731	2560	(93.7%)	171	(6.3%)	1.00		1.00	
Hypospadias isolated	341	313	(91.8%)	28	(8.2%)	1.34	(0.88-2.03)	1.35	(0.88-2.08)
Hypospadias co-occurring with other CAs	30	<5				NC			
Pesticides									
Controls [b]	2731	2685	(98.3%)	46	(1.7%)	1.00		1.00	
Hypospadias isolated	341	336	(98.5%)	5	(1.5%)	0.87	(0.34-2.20)	0.84	(0.32-2.17)
Hypospadias co-occurring with other CAs	30	<5				NC			
Organic Solvents/ Alkylphenolic compounds[c]									
Controls [b]	2731	2560	(93.7%)	171	(6.3%)	1.00		1.00	
Hypospadias isolated	341	320	(93.8%)	21	(6.2%)	0.97	(0.61-1.55)	0.88	(0.54-1.43)
Hypospadias co-occurring with other CAs	30	<5				NC			
Phthalates/Benzophenones/ Parabens/Siloxanes[c]									
Controls [b]	2731	2620	(95.9%)	111	(4.1%)	1.00		1.00	
Hypospadias isolated	341	351	(94.6%)	20	(5.4%)	1.35	(0.83-2.19)	1.21	(0.73-2.01)
Hypospadias co-occurring with other CAs	3	<5				NC			

[a] adjusted for birth year, maternal age and body mass index, smoking and alcohol use during pregnancy, [b] only boys are selected as controls. [c] exposure to at least one exposure in this group. EDCs = endocrine disrupting chemicals, PAH = polycyclic aromatic hydrocarbons, NC = not calculated due to sparse data (<5 exposed cases), CAs = congenital anomalies.

Supplementary Table 7 | Prevalence, crude OR, and adjusted OR of probable maternal occupational exposed to EDCs and the risk of hypospadias in the offspring (Eurocat) compared to non-malformed controls (Lifelines), North Netherlands, 1997-2013

	Total	Unexposed		Exposed		Unadjusted		Adjusted [a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Any EDC									
Controls [b]	2369	2214	(93.5%)	155	(6.5%)	1.00		1.00	
Hypospadias	310	286	(92.3%)	24	(7.7%)	1.20	(0.77-1.88)	1.10	(0.69-1.77)
PAHS									
Controls [b]	2620	2560	(97.7%)	60	(2.3)	1.00		1.00	
Hypospadias	354	340	(96.0%)	14	(4.0%)	1.76	(0.97-3.18)	1.57	(0.84-2.95)
Pesticides									
Controls [b]	2716	2685	(98.9%)	31	(1.1%)	1.00		1.00	
Hypospadias	369	<5				NC		NC	
Organic Solvents/ Alkylphenolic compounds[c]									
Controls [b]	2561	2558	(99.9%)	3	(0.1%)	1.00		1.00	
Hypospadias	320	<5				NC		NC	
Phthalates/Benzophenones/ Parabens/Siloxanes[c]									
Controls [b]	2697	2620	(97.1%)	77	(2.9%)	1.00		1.00	
Hypospadias	338	322	(95.3%)	16	(4.7%)	1.69	(0.98-2.93)	1.22	(0.91-1.63)

[a] adjusted for birth year, maternal age and body mass index, smoking and alcohol use during pregnancy. [b] only boys are selected as controls. [c] exposure to at least one exposure in this group. EDCs = endocrine disrupting chemicals, PAH = polycyclic aromatic hydrocarbons, NC = not calculated due to sparse data (<5 exposed cases).

Supplementary Table 8 | Prevalence, crude OR, and adjusted OR of maternal occupational exposure to EDCs and the risk of hypospadias in the offspring (Eurocat) compared to non-malformed controls (males only) and non-exposed as reference category (Lifelines), North Netherlands, 1997-2013

	Total	Unexposed		Exposed		Unadjusted		Adjusted [a]	
Occupational exposure	n	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Any EDC									
Controls [b]	2369	2214	(93.5%)	155	(6.5%)	1.00		1.00	
Hypospadias	310	286	(92.3%)	24	(7.7%)	1.20	(0.77-1.88)	1.10	(0.69-1.77)
PAHS									
Controls [b]	2385	2214	(92.8%)	171	(7.2%)	1.00		1.00	
Hypospadias	317	286	(90.2%)	31	(9.8%)	1.40	(0.94-2.10)	1.42	(0.93-2.16)
Pesticides									
Controls [b]	2260	2214	(98.0%)	46	(2.0%)	1.00		1.00	
Hypospadias	291	286	(98.3%)	5	(1.7%)	0.84	(0.33-2.14)	0.81	(0.31-2.10)
Organic Solvents/ Alkylphenolic compounds[c]									
Controls [b]	2387	2214	(92.8%)	173	(7.2%)	1.00		1.00	
Hypospadias	310	286	(92.3%)	24	(7.7%)	1.07	(0.69-1.68)	0.99	(0.62-1.57)
Phthalates/Benzophenones/ Parabens/Siloxanes[c]									
Controls [b]	2325	2214	(95.2%)	111	(4.8%)	1.00		1.00	
Hypospadias	306	286	(93.5%)	20	(6.5%)	1.40	(0.85-2.28)	1.26	(0.75-2.11)

[a] adjusted for birth year, maternal age and body mass index, smoking and alcohol use during pregnancy. [b] only boys are selected as controls. [c] exposure to at least one exposure in this group. EDCs = endocrine disrupting chemicals, PAH = polycyclic aromatic hydrocarbons, NC = not calculated due to sparse data (<5 exposed cases).

CHAPTER 5

Maternal occupational exposure and congenital heart defects in offspring

Nynke Spinder
Jorieke E.H. Bergman
Hans Kromhout
Roel C.H. Vermeulen
Nicole Corsten-Janssen
H. Marike Boezen
Gideon J. du Marchie Sarvaas
Hermien E.K. de Walle

Scandinavian Journal of Work, Environment & Health. 2020; online first

ABSTRACT

Objective Congenital heart defects (CHDs) are the most prevalent congenital anomalies. This study aims to examine the association between maternal occupational exposures to organic and mineral dust, solvents, pesticides, and metal dust and fumes and CHDs in the offspring, assessing several subgroups of CHDs.

Methods For this case-control study, we examined 1,174 cases with CHDs from Eurocat Northern Netherlands and 5,602 controls without congenital anomalies from the Lifelines cohort study. Information on maternal jobs held early in pregnancy was collected via self-administered questionnaires, and job titles were linked to occupational exposures using a job exposure matrix.

Results An association was found between organic dust exposure and coarctation of aorta (adjusted odds ratio [aOR] 1.90, 95% Confidence Interval [95%CI] 1.01-3.59) and pulmonary (valve) stenosis in combination with ventricular septal defect (aOR 2.68, 95%CI 1.07-6.73). Mineral dust exposure was associated with increased risk of coarctation of aorta (aOR 2.94, 95%CI 1.21-7.13) and pulmonary valve stenosis (aOR 1.99, 95%CI 1.10-3.62). Exposure to metal dust and fumes was infrequent, but was associated with CHDs in general (aOR 2.40, 95%CI 1.09-5.30). Exposure to both mineral dust and metal dust and fumes was associated with septal defects (aOR 3.23, 95%CI 1.14-9.11). Any maternal occupational exposure was associated with a lower risk of aortic stenosis (aOR 0.32, 95%CI 0.11-0.94).

Conclusion Women should take preventive measures or avoid exposure to mineral dust, organic dust, and metal dust and fumes early in pregnancy since this could affect foetal heart development.

INTRODUCTION

Congenital heart defects (CHDs) are the most prevalent congenital anomalies. Approximately 7 per 1,000 pregnancies are affected by a CHD [1]. Of these, >90% are live births, ~8% of the pregnancies are terminated because of CHDs, and 1-2% are still births [1]. Since the introduction of prenatal ultrasound screening, ~50% of critical CHD cases are detected prenatally, and this number continues to increase with improvements in ultrasound technology, recommendations, and training for foetal heart examination [2]. Survival rates are also increasing due to improved surgical intervention and intensive care [3]. Major CHDs have a significant impact on children's physical and mental health in the short- and long-term [4,5], making it important to identify modifiable risk factors to prevent CHDs in offspring.

Both genetic and environmental factors are involved in the development of CHDs. Chromosomal anomalies are found in 12% of the infants with CHDs [6], and an increasing number of gene point mutations have been identified that cause isolated non-syndromic CHD [7]. Having first-degree family members with CHDs or a multiple pregnancy increases the risk of CHDs in offspring by 1-10% [8]. In addition, certain maternal illnesses (e.g. maternal diabetes, phenylketonuria, rubella infection), exposure to specific medications during pregnancy (e.g. anticonvulsants and higher doses of lithium), and high maternal weight increase the risk of CHD in offspring [8,9]. Lifestyle factors such as parental smoking and alcohol use can also increase the risk of CHDs [8-10], while periconceptional folic acid supplementation decreases this risk [11]. Other risk factors are exposure to environmental agents such as ambient air pollution, chemicals, and metals [12,13].

Exposure to potential teratogenic agents can occur in the workplace. A recent meta-analysis found an association between maternal occupational exposure to solvents and CHDs [14]. In this meta-analysis, it was not possible to examine subgroups of CHDs since the majority of studies selected included small numbers of cases. However, it is important to assess subgroups of CHDs, as defects differ in aetiology and develop during different stages of embryogenesis. The aim of the present study is to examine the association between various types of maternal occupational exposures early in pregnancy and subgroups of CHDs in the offspring.

METHODS

Study design

Cases were selected from the European Concerted Action on Congenital Anomalies and Twins Northern Netherlands (Eurocat NNL). This registry collects data of infants born with a congenital anomaly in the three northern provinces of the Netherlands. In addition to live-born infants (up to 10 years of age at notification), Eurocat NNL registers stillbirths, miscarriages, and terminated pregnancies affected by a congenital anomaly. Eurocat NNL identifies eligible cases by active case ascertainment using hospital records, prenatal diagnosis records, and postmortem records. After parents give informed consent, they are asked to complete a questionnaire. Information is collected regarding the pregnancy, obstetric and medical history, demographic characteristics, use of medication, and occupation and lifestyle factors early in pregnancy [15].

Controls without congenital anomalies (non-malformed controls) were selected from the Lifelines cohort. Lifelines is a three-generation cohort study following 167,000 participants over a 30-year period in the same geographical region as Eurocat NNL. Lifelines participants were recruited through their general practitioners, and participants (between 18 and 65 years old) were also asked to invite their offspring and parents in order to create a three-generation cohort. Participant's children could participate if they were between 6 months and 18 years old. Parents of participating children completed a questionnaire regarding the pregnancy, their health during pregnancy, childbirth, and the child's health in the first six months of life [16].

Case and control definition

CHD cases were coded by trained registry staff according to the International Classification of Diseases 9th revision (ICD-9) until 2001 and according to ICD 10th revision (ICD-10) from 2002 onwards, using international EUROCAT guidelines [17,18]. Cases with heterotaxy syndrome or an underlying genetic, chromosomal, or syndromic condition were excluded, resulting in the selection of 1,922 CHD cases born between 1997 and 2013 (Figure 1). Mothers with missing job information (n=400) or without a job (n=260) were excluded to avoid healthy worker bias.

The remaining cases were classified according to the Botto classification by three of the study authors (NS, JB, and GMS) to account for the diversity of cardiac phenotypes and underlying developmental mechanisms. The Botto classification has been described

previously [19]. Briefly, morphologically homogeneous groups were produced for each cardiac phenotype, based on anatomy and developmental and epidemiologic evidence. The seven main heart defect groups were: conotruncal heart defects, atrioventricular septal defects (AVSD), anomalous pulmonary venous return (APVR), left ventricular outflow tract obstruction (LVOTO), right ventricular outflow tract obstruction (RVOTO), septal defects, and complex heart defects. A few cardiac malformations are not included in the Botto classification. In line with the classification described by Riehle-Collarusso and colleagues [20], cases with a bicuspid aorta valve were classified as LVOTO anomaly and cases with a vascular ring (vascular rings/slings, double aortic arch, right descending aortic arch, aberrant left subclavian artery, or pulmonary artery sling) were classified as conotruncal defects. Cases were excluded if they could not be classified (e.g. coronary artery malformations, n=52) or constituted isolated patent ductus arteriosus (n=24). Additionally, CHDs were classified as isolated defect (only the heart is affected) or as multiple defect (presence of cardiac and extra-cardiac malformations). Cases were also classified by the complexity of their cardiac phenotype: simple (anatomically discrete or well-recognized single entities), association (common, uncomplicated combinations of heart defects), and complex malformations (those not described as simple or association). If multiple siblings were affected by a CHD, one infant per family was randomly selected to avoid genetic correlation, resulting in exclusion of 12 cases. Overall, 1,174 infants with CHDs were included.

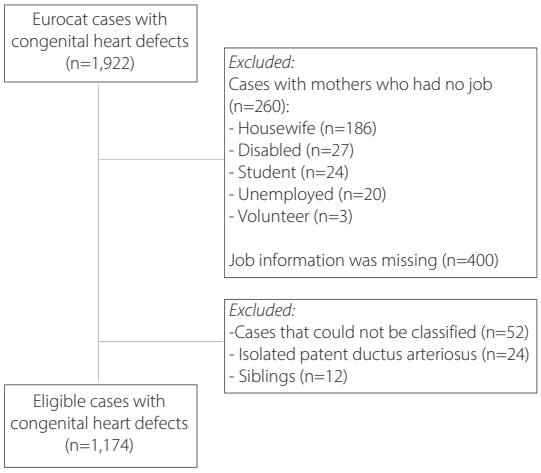


Figure 1 | Flowchart case selection from Eurocat North Netherlands

As controls, we selected 12,494 participants from the Lifelines cohort born between 1997 and 2013 (same years as the Eurocat NNL cases)(Figure 2). Only infants of which the biological mother was a Lifelines participant were included (n=12,331). We excluded 814 infants because one or more congenital anomalies were reported, or information on congenital anomalies was missing. As with cases, mothers without a job or missing job information were excluded (n=3,029) and only one infant per family was selected, resulting in exclusion of another 2,886 infants. In total, 5,602 children without congenital anomalies were included as control group.

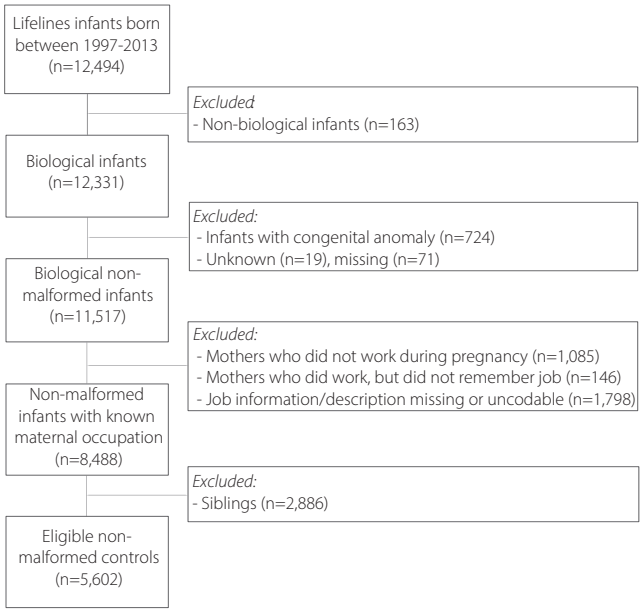


Figure 2 | Flowchart control selection from Lifelines

Exposure assessment

The mother's description of her job early in pregnancy was coded by two authors (NS, HK) using the International Standard Classification of Occupations 1988 (ISCO88) [21], without knowledge of case or study details. To translate ISCO88 codes into occupational exposure, the ALOHA+ Job Exposure Matrix (JEM) was used. Occupational exposure was assigned based on six categories: organic and mineral dust, solvents, pesticides, metal dust and fumes, and gases and fumes. This JEM assigns exposure intensity in three categories (no, low, and high exposure). Because "high" (intensity and probability) exposure did not occur often, the categories "low" and "high" were combined into "exposed". The ALOHA+

JEM is specifically built for use in general population studies [22,23]. However, in our female study population there was a strong correlation of exposure to solvents with exposure to gases and fumes and to organic dust with gases and fumes (Spearman's rank correlation coefficient = 0.75 and 0.80, respectively). Therefore, the association of gases and fumes with CHD was not analysed.

Statistical analysis

Baseline characteristics of mothers and infants were tabulated, and differences between cases and controls were tested for significance using Chi-square tests. The following covariates were assessed: child sex (male/female), birth year (1997-2000, 2001-2004, 2005-2008, or 2009-2013), maternal age at delivery (15-19, 20-24, 25-29, 30-34, 35-39, or ≥40 years old), maternal body mass index (BMI) (self-reported pre-pregnancy weight and height for Eurocat NNL cases and objective measurement at baseline visit for Lifelines controls)(underweight [<18.5 kg/m^2], normal [18.5-24.9 kg/m^2], overweight [25.0-29.9 kg/m^2], or obese [≥30 kg/m^2]), maternal education level (low [primary school, lower vocational education, pre-vocational education], middle [secondary vocational education, general secondary education or pre-university education], or high [higher professional education or academic education]), maternal smoking and alcohol use, folic acid use (no/not during periconceptional period, yes/sometime during periconceptional period), and fertility problems (no, yes [self-reported fertility problems and/or fertility treatment]).

The association between maternal occupational exposure early in pregnancy and CHDs was assessed using univariate and multivariate logistic regression analysis to estimate crude odds ratios (ORs) and adjusted odds ratios (aORs). The multivariate logistic regression associations were adjusted for child sex, maternal age at delivery, maternal educational level, maternal BMI, smoking and alcohol use during pregnancy, folic acid supplementation, and fertility problems, based on Chi-square tests (Table 1). Although the correlation between exposure to mineral dust and exposure to metal dust and fumes was negligible (Spearman's rank correlation coefficient = 0.08), exposure to metal dust and fumes contributes to mineral dust exposure. Consequently, additional analyses were performed with a combination of those exposures. Stratified analyses were performed for cases with isolated and multiple defects. An exposure–response analysis was conducted for maternal occupational exposure and CHDs in general. If <5 infants were exposed, data was not presented and ORs were not estimated.

RESULTS

Baseline characteristics differed between cases and controls (Table 1). Infants born with a CHD were more often boys. Mothers of case infants had a lower maternal age at delivery, lower educational level, and lower BMI. As expected they were also more likely to smoke or consume alcohol, used folic acid supplements less often, and had more fertility problems compared to mothers of controls.

Table 1 | Baseline characteristics of Lifelines controls and Eurocat cases

	Controls (n=5602)		CHDs (n=1174)		p-value
	n	(%)	n	(%)	
Child sex					<0.01
Male	2731	(48.8%)	632	(53.8%)	
Female	2871	(51.2%)	542	(46.2%)	
Birth year					0.12
1997-2000	1240	(22.1%)	266	(22.8%)	
2001-2004	1660	(29.6%)	310	(26.1%)	
2005-2008	1293	(23.1%)	298	(25.5%)	
2009-2013	1409	(25.2%)	300	(25.6%)	
Maternal age at delivery					<0.01
15-19[a]	3	(0.1%)	1	(0.1%)	
20-24	191	(3.6%)	100	(8.7%)	
25-29	1492	(28.2%)	362	(31.6%)	
30-34	2470	(46.7%)	479	(16.2%)	
35-39	1058	(20.0%)	194	(16.9%)	
≥40	73	(1.4%)	11	(1.0%)	
Unknown	315		27		
Education level					<0.01
Low	649	(12.3%)	162	(14.0%)	
Middle	2396	(45.4%)	561	(48.6%)	
High	2236	(42.3%)	432	(37.4%)	
Unknown	321		19		
Body mass index (kg/m^2)[b]					<0.01
<18.5	56	(1.0%)	31	(2.7%)	
18.5-24.9	2871	(53.6%)	738	(64.6%)	
25.0-29.9	1610	(30.1%)	269	(23.5%)	
≥30	818	(15.3%)	105	(9.2%)	
Unknown	247		32		

Table 1. Continued

	Controls (n=5602)		CHDs (n=1174)		p-value
	n	(%)	n	(%)	
Smoking during first trimester					<0.01
No	5036	(90.2%)	903	(77.2%)	
Yes	549	(9.8%)	267	(22.8%)	
Unknown	17		4		
Alcohol during first trimester					<0.01
No	5045	(90.3%)	873	(74.6%)	
Yes	544	(9.7%)	297	(25.4%)	
Unknown	13		4		
Folic acid use					<0.01
No	847	(16.5%)	290	(24.9%)	
Yes	4272	(83.5%)	873	(75.1%)	
Unknown	483		11		
Fertility problems					<0.01
No	5230	(93.9%)	971	(83.6%)	
Yes	339	(6.1%)	190	(16.4%)	
Unknown	33		13		

[a]Lifelines includes participants from 18 years old. [b]Body mass index of Eurocat cases is based on self-reported height and weight. Height and weight of Lifelines participants is measured at the baseline visit to the study clinic.

In total, 37.6% of CHD infants and 35.6% of the control infants were exposed to any of the maternal occupational exposures early in pregnancy (Table 2), and no association was found between any exposure and CHDs in general. When examining any exposure and specific groups of CHDs, we found an association for pulmonary (valve) stenosis in combination with ventricular septal defect (VSD) (aOR 3.06, 95%CI 1.20-7.81). However, any exposure is also associated with a lower risk of aortic stenosis (aOR 0.32, 95%CI 0.11-0.94).

When analysing specific exposures, the most prevalent maternal occupational exposure was to organic dust, with approximately 30% of women exposed. Associations were found between organic dust exposure and coarctation of aorta (aOR 1.90, 95%CI 1.01-3.59) and pulmonary (valve) stenosis in combination with VSD (aOR 2.68, 95%CI 1.07-6.73). Mineral dust exposure was less common (10% of cases and 8% of controls) and was associated with CHDs in general (aOR 1.29, 95%CI 1.01-1.64). When analysing mineral dust exposure in relation to specific CHDs, we found an association with LVOTO defects (aOR 1.75, 95%CI 1.06-2.89), particularly coarctation of the aorta (aOR 2.94, 95%CI 1.21-7.13), and with RVOTO defects, especially pulmonary (valve) stenosis (aOR 1.99, 95%CI 1.10-3.62).

Approximately 25% of mothers were exposed to solvents and 2-3% to pesticides, but no associations between exposure to solvents or pesticides and CHDs were found. Although the prevalence of exposure to metal dust and fumes was only 0.4% for controls and 1% for cases, we did observe an association between this exposure and CHDs in general (aOR 2.40, 95%CI 1.09-5.30). When mothers were exposed to mineral dust and metal dust and fumes, the association with CHDs in general became stronger compared to exposure to mineral dust or metal dust and fumes alone (aOR 2.92, 95%CI 1.23-6.92), and an association with septal defects was found (aOR 3.23, 95%CI 1.14-9.11) (Supplementary Table 1).

Stratified analysis by isolated and multiple defects included 1,009 cases with isolated CHDs and 165 cases with CHDs and extra-cardiac malformations. The aORs for isolated CHDs were comparable to the total group of CHDs (Supplementary Table 2). One additional association was observed when only isolated defects were included: exposure to metal dust and fumes was associated with septal defects (aOR 3.06, 95%CI 1.14-8.23). The aORs for multiple defects that include CHDs showed no association for any of the exposures (Supplementary Table 3). Only a small number of cases were included in the stratified analyses for multiple defects, and most aORs were not estimated due to sparse outcome and exposure data.

An exposure–response analysis was performed for any exposure and CHDs in general. The aOR appeared to be non-significant but higher in the high exposure group only (aOR 1.37, 95%CI 0.97-1.94; Supplementary Table 4).

Table 2 | Prevalence, crude OR and adjusted OR of maternal occupational exposure and CHDs in the offspring.

		Occupational exposure																			
		Any exposure						Organic dust						Mineral dust							
	Total	Exposed		Unadjusted		Adjusted [a]		Exposed		Unadjusted		Adjusted [a]		Exposed				Unadjusted		Adjusted [a]	
CHD classification	n	n	%	OR	95% CI	OR	95% CI	n	%	OR	95% CI	OR	95% CI	n	%	OR	95% CI	OR		95% CI	
Controls	5602	1992	(35.6%)	Ref		Ref	1992	1617	(28.9%)	Ref		Ref		418	(7.5%)	Ref		Ref			
Total CHD	1174	442	(37.6%)	1.09	0.96-1.25	1.04	0.90-1.20	356	(30.3%)	1.07	0.94-1.23	1.10	0.95-1.28	120	(10.2%)	**1.41**	**1.14-1.75**	**1.29**		**1.01-1.64**	
Conotruncal	174	69	(39.7%)	1.19	0.88-1.62	1.13	0.81-1.57	57	(32.8%)	1.20	0.87-1.66	1.30	0.93-1.82	18	(10.3%)	1.43	0.87-2.36	1.31		0.76-2.26	
d-TGA	74	28	(37.8%)	1.10	0.69-1.77	1.00	0.60-1.68	23	(31.1%)	1.11	0.68-1.82	1.18	0.70-1.99	6	(8.1%)	1.09	0.47-2.54	0.95		0.37-2.46	
Tetralogy of Fallot	60	28	(46.7%)	1.59	0.95-2.64	1.50	0.88-2.57	23	(38.3%)	1.53	0.91-2.59	1.68	0.98-2.89	8	(13.3%)	1.91	0.90-4.04	1.77		0.80-3.94	
Truncus arteriosus	10	5	(50.0%)	1.81	0.52-6.27	1.46	0.41-5.24	<5		NC		NC		<5		NC		NC			
LVOTO	173	62	(35.8%)	1.01	0.74-1.39	0.94	0.67-1.31	53	(30.6%)	1.09	0.78-1.51	1.14	0.80-1.60	22	(12.7%)	**1.81**	**1.14-2.86**	**1.75**		**1.06-2.89**	
HLHS	50	19	(38.0%)	1.11	0.63-1.97	0.86	0.47-1.57	15	(30.0%)	1.06	0.58-1.94	0.87	0.46-1.67	7	(14.0%)	2.02	0.90-4.52	1.51		0.62-3.72	
Aortic stenosis	31	5	(16.1%)	**0.35**	**0.13-0.91**	**0.32**	**0.11-0.94**	<5		NC		NC		<5		NC		NC			
Coarctation of aorta	42	18	(42.9%)	1.36	0.74-2.51	1.33	0.70-2.54	17	(40.5%)	1.68	0.90-3.11	**1.90**	**1.01-3.59**	7	(16.7%)	**2.48**	**1.10-5.62**	**2.94**		**1.21-7.13**	
Bicuspid aortic valve	42	16	(38.1%)	1.12	0.60-2.08	1.14	0.59-2.18	14	(33.3%)	1.23	0.65-2.35	1.37	0.70-2.65	5	(11.9%)	1.68	0.66-4.29	1.56		0.58-4.18	
RVOTO	139	58	(41.7%)	1.30	0.92-1.83	1.24	0.87-1.77	49	(35.3%)	1.34	0.94-1.91	1.35	0.94-1.95	19	(13.7%)	**1.96**	**1.20-3.22***	**1.75**		**1.02-3.00**	
P(v)S	104	44	(42.3%)	1.33	0.90-1.97	1.26	0.84-1.90	37	(35.6%)	1.36	0.91-2.04	1.36	0.89-2.07	16	(15.2%)	**2.26**	**1.31-3.88***	**1.99**		**1.10-3.62**	
Pulmonary atresia	13	6	(46.2%)	1.55	0.52-4.63	1.37	0.45-4.20	6	(46.2%)	2.11	0.71-6.30	2.13	0.70-6.43	<5		NC		NC			
Septal	544	194	(35.7%)	1.01	0.84-1.21	0.96	0.79-1.17	150	(27.6%)	0.94	0.77-1.14	0.97	0.79-1.19	48	(8.8%)	1.20	0.88-1.64	1.06		0.75-1.49	
Perimembranous VSD	117	51	(43.6%)	1.40	0.97-2.03	1.34	0.91-1.99	40	(34.2%)	1.28	0.87-1.88	1.40	0.94-2.10	12	(10.3%)	1.42	0.77-2.60	1.31		0.69-2.50	
Muscular VSD	248	79	(31.9%)	0.85	0.65-1.11	0.87	0.65-1.16	63	(25.4%)	0.84	0.63-1.12	0.90	0.67-1.22	21	(8.5%)	1.15	0.73-1.82	1.15		0.71-1.88	
Other VSD	78	27	(34.6%)	0.96	0.60-1.53	0.98	0.60-1.61	18	(23.1%)	0.74	0.44-1.26	0.82	0.47-1.41	7	(9.0%)	1.22	0.56-2.68	1.11		0.48-2.53	
ASD	98	36	(36.7%)	1.05	0.70-1.59	0.93	0.60-1.43	28	(28.6%)	0.99	0.63-1.53	0.95	0.60-1.51	8	(8.2%)	1.10	0.53-2.29	0.83		0.38-1.80	
AVSD	28	7	(25.0%)	0.60	0.26-1.42	0.67	0.27-1.63	6	(21.4%)	0.67	0.27-1.66	0.81	0.32-2.06	<5		NC		NC			
APVR	17	9	(52.9%)	2.04	0.79-5.29	1.88	0.69-5.10	8	(47.1%)	2.19	0.84-5.69	1.99	0.73-5.41	<5		NC		NC			
Total APVR	11	5	(45.5%)	1.51	0.46-4.96	1.48	0.44-4.97	5	(45.5%)	2.05	0.63-6.74	2.03	0.61-6.76	<5		NC		NC			
Complex	45	19	(42.2%)	1.32	0.73-2.40	1.30	0.68-2.47	16	(35.6%)	1.36	0.74-2.51	1.39	0.72-2.70	<5		NC		NC			
Single ventricle	14	8	(57.1%)	2.42	0.84-6.97	2.54	0.80-8.11	6	(42.9%)	1.85	0.64-5.34	2.13	0.69-6.54	<5		NC		NC			
Associations																					
CoA + VSD	15	7	(46.7%)	1.59	0.57-4.38	1.69	0.59-4.83	5	(33.3%)	1.23	0.42-3.61	1.36	0.56-4.08	<5		NC		NC			
P(v)S + VSD	19	11	(57.9%)	**2.49**	**1.00-6.21**	**3.06**	**1.20-7.81**	9	(47.4%)	2.22	0.90-5.47	**2.68**	**1.07-6.73**	<5		NC		NC			

Table 2. Continued

		Occupational exposure																	
		Solvents						Pesticides						Metal dust and fumes					
	Total	Exposed		Unadjusted		Adjusted [a]		Exposed		Unadjusted		Adjusted [a]		Exposed				Unadjusted	Adjusted [a]
CHD classification	n	n	%	OR	95% CI	OR	95% CI	n	%	OR	95% CI	OR	95% CI	n	%	OR	95% CI	OR	95% CI
Controls	5602	1370	(24.5%)	Ref		Ref		131	(2.3%)	Ref		Ref		20	(0.4%)	Ref		Ref	
Total CHD	1174	275	(23.4%)	0.95	0.82-1.10	0.95	0.81-1.11	34	(2.9%)	1.25	0.85-1.83	1.20	0.79-1.81	12	(1.0%)	**2.88**	**1.41-5.91**	**2.40**	**1.09-5.30**
Conotruncal	174	40	(23.0%)	0.92	0.64-1.32	1.00	0.69-1.45	<5		NC		NC		<5		NC		NC	
d-TGA	74	16	(21.6%)	0.85	0.49-1.49	0.98	0.55-1.73	<5		NC		NC		<5		NC		NC	
Tetralogy of Fallot	60	18	(30.0%)	1.32	0.76-2.31	1.38	0.78-2.43	<5		NC		NC		<5		NC		NC	
Truncus arteriosus	10	<5		NC		NC		<5		NC		NC		<5		NC		NC	
LVOTO	173	37	(21.4%)	0.84	0.58-1.22	0.81	0.55-1.20	7	(4.0%)	1.76	0.81-3.83	1.73	0.77-3.87	<5		NC		NC	
HLHS	50	11	(22.0%)	0.87	0.45-1.71	0.75	0.37-1.52	<5		NC		NC		<5		NC		NC	
Aortic stenosis	31	<5		NC		NC		<5		NC		NC		<5		NC		NC	
Coarctation of aorta	42	8	(19.0%)	0.73	0.34-1.57	0.74	0.34-1.63	<5		NC		NC		<5		NC		NC	
Bicuspid aortic valve	42	11	(26.2%)	1.10	0.55-2.19	1.18	0.58-2.40	<5		NC		NC		<5		NC		NC	
RVOTO	139	37	(26.6%)	1.12	0.77-1.64	1.13	0.76-1.66	5	(3.6%)	1.56	0.63-3.87	1.45	0.57-3.67	<5		NC		NC	
P(v)S	104	26	(25.0%)	1.03	0.66-1.61	1.05	0.66-1.65	4	(3.8%)	NC		NC		<5		NC		NC	
Pulmonary atresia	13	<5		NC		NC		<5		NC		NC		<5		NC		NC	
Septal	544	121	(22.2%)	0.88	0.72-1.09	0.90	0.72-1.13	16	(2.9%)	1.27	0.75-2.14	1.20	0.69-2.09	6	(1.1%)	**3.11**	**1.25-7.78**	2.47	0.92-6.64
Perimembranous VSD	117	32	(27.4%)	1.16	0.77-1.75	1.24	0.81-1.89	5	(4.3%)	1.86	0.75-4.64	1.80	0.70-4.61	<5		NC		NC	
Muscular VSD	248	52	(21.0%)	0.82	0.60-1.12	0.84	0.61-1.16	5	(2.0%)	0.86	0.35-2.12	0.87	0.35-2.20	<5		NC		NC	
Other VSD	78	12	(15.4%)	0.56	0.30-1.04	0.60	0.32-1.12	<5		NC		NC		<5		NC		NC	
ASD	98	24	(24.5%)	1.00	0.63-1.59	0.97	0.60-1.58	<5		NC		NC		<5		NC		NC	
AVSD	28	<5		NC		NC		<5		NC		NC		<5		NC		NC	
APVR	17	8	(47.1%)	**2.75**	**1.06-7.13**	2.18	0.80-5.93	<5		NC		NC		<5		NC		NC	
Total APVR	11	5	(45.5%)	2.57	0.78-8.45	2.23	0.67-7.42	<5		NC		NC		<5		NC		NC	
Complex	45	15	(33.3%)	1.55	0.83-2.88	1.58	0.82-3.07	<5		NC		NC		<5		NC		NC	
Single ventricle	14	5	(35.7%)	1.72	0.57-5.13	1.97	0.62-6.20	<5		NC		NC		<5		NC		NC	
Associations																			
CoA + VSD	15	<5		NC		NC		<5		NC		NC		<5		NC		NC	
P(v)S + VSD	19	7	(36.8%)	1.80	0.71-4.59	1.82	0.70-4.73	<5		NC		NC		<5		NC		NC	

CHD, congenital heart defects; d-TGA, dextro-transposition of the great arteries; LVOTO, left ventricular outflow tract obstruction; HLHS, hypoplastic left heart syndrome; RVOTO, right ventricular outflow tract obstruction; P(v)S, pulmonary (valve) stenosis; CoA= coarctation of aorta; VSD, ventricular septal defect; ASD, atrial septal defect; AVSD, atrioventricular septal defect; APVR, anomalous pulmonary venous return; NC, not calculated due to sparse data. [a] adjusted for child sex, maternal age at delivery (as continuous variable), education level, maternal BMI (as continuous variable), smoking and alcohol use during pregnancy, folic acid supplementation, and fertility problems. *p-value <0.01.

DISCUSSION

This study showed that infants with specific CHDs were more likely to be exposed in utero to organic dust, mineral dust, and metal dust and fumes at the workplace of mother compared with infants without malformations. Exposure to organic dust was associated with a two-fold increased risk of coarctation of aorta and a three-fold increased risk of pulmonary (valve) stenosis in combination with VSD. This exposure occurs most often in personal care workers, nursing professionals, and cleaners. Mineral dust exposure was associated with a two-fold increase in LVOTO defects in offspring, specifically coarctation of the aorta, and RVOTO defects, specifically pulmonary (valve) stenosis. Cleaners and agricultural workers are those most likely to be exposed to mineral dust. Exposure to metal dust and fumes was associated with a two-fold increase of CHDs in general. However, this result has to be interpreted carefully as only 1% of the women, mostly those working as machine and instrument operators/repairers, were occupationally exposed to metal dust and fumes. Exposure to mineral dust in combination with metal dust and fumes was associated with a three-fold increased risk of septal defects. We also found that infants affected by aortic stenosis were less likely to be exposed to any maternal occupational exposure compared to non-malformed controls. However, only five cases with aortic stenosis were included, and analyses for specific subgroups of exposure could not be performed. No specific job association was identified.

During their work, mothers may inhale mineral, metal or organic aerosols, which can pass through the lungs into the blood. These agents might consequently cross the placental barrier and have been found at the foetal side of the placenta[24]. Occupational exposures, including to several organic, mineral, and metal compounds, can induce oxidative stress, which may induce teratogenesis via misregulation of critical pathways involved in foetal development [25].

Although the association between metal dust and fumes and CHDs/septal defects has to be interpreted with caution, previous studies found increased risks. One study found an association between exposure to metals and specific septal defects [26]. Two other studies showed that maternal occupational exposure to mineral oils, which are often used in the metal industry, increased the risk of isolated septal defects [27] and coarctation of the aorta [28]. Another study using comparable methods did not show this association, but these estimates could have been imprecise as this study included <5 exposed cases [29]. To our knowledge, no studies specifically examining organic or mineral dust have been reported.

Our results did not confirm the association between occupational exposure to solvents and CHDs reported by a meta-analysis using similar occupational exposure assessment methods [14]. It is possible that the difference is explained by the diversity of CHDs included in the meta-analysis. One previous study assessing solvent exposure and specific types of CHDs also showed no association [30], but another study found an association with perimembranous VSD and aorta stenosis [31]. Our results on maternal occupational exposure to pesticides are in line with the meta-analysis, which also found no association with CHDs [14]. One previous study found an association between pesticide exposure and specific CHDs, such as RVOTO defects, hypoplastic left heart syndrome, and tetralogy of Fallot [32]. Unfortunately, our sample size was too limited to analyse these specific CHDs.

Limitations

Occupational exposure assessment using the ALOHA+ JEM is done at job level, which could have resulted in misclassification of exposure. Circumstances at the workplace are often unpredictable and can vary within jobs, between workplaces and over time [33]. It is also possible that women avoided certain exposures because they wanted to become pregnant or knew they were pregnant while performing a job that would normally come with these exposures. Moreover, the limited number of exposed women restricted our ability to explore exposure–response associations for specific exposures or specific CHDs.

Because Eurocat NNL does not collect data on non-malformed controls, controls were selected from Lifelines, and this approach introduced several limitations. Eurocat NNL aims to investigate the prevalence and risk factors for congenital anomalies, and their questionnaire is focused specifically on risk factors for congenital anomalies. Lifelines collects data to obtain insight into healthy ageing, and specifically for children on neonatal and childhood diseases. Consequently, the Lifelines questionnaire includes items on a wide variety of risk factors. These differences could introduce information bias during assessment of the covariates. We assume that bias was not introduced for maternal occupational exposure, as mothers were asked to report a description of their job early in pregnancy in both questionnaires and recall bias is limited for self-reported jobs [33]. Additionally, residual confounding due to maternal diabetes, paternal smoking, or environmental exposures could have been introduced since information regarding those risk factors was lacking.

Another major concern of using Lifelines is selection bias. Previous studies showed that some groups of individuals, for example those with a low socioeconomic status, are less likely to participate in population-based cohort studies [34,35]. However, Lifelines is known

to be representative for the population in the Northern Netherlands, indicating selection bias might be low [36].

Strengths

A major strength of this study is the high quality of data from Eurocat NNL, which registers detailed medical information for each case. Anomalies were coded by trained registry staff according to international coding guidelines [18]. Case classification was performed under supervision of an experienced clinical geneticist and a paediatric cardiologist. Use of the Botto classification made it possible to create homogenous groups of CHDs based on anatomy and developmental and epidemiological evidence [19]. Another strength is that Eurocat identifies eligible cases by active case ascertainment using various sources in the catchment area, and ~72% of the parents of a child affected by a CHD agreed to participate and responded to the questionnaire. A major strength of the JEM approach is that it limits the effect of recall bias on exposure status as well as differential misclassification of exposure when compared to self-reported exposure [22,37].

Conclusion

This large population-based case-control study shows that maternal occupational exposure to organic dust, mineral dust, and metal dust and fumes early in pregnancy could affect the development of the foetal heart. These exposures, with a prevalence of 1-30% at the workplace, were associated with a two- to three-fold increase in LVOTO, RVOTO, and septal defects. Women should avoid exposure to mineral and organic dusts and metal dust and fumes in the months before and early in pregnancy.

REFERENCES

1. EUROCAT. Prevalence charts and tables. https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-data/prevalence. Updated 2017. Accessed 1/15, 2020.
2. Bakker MK, Bergman JEH, Krikov S, et al. Prenatal diagnosis and prevalence of critical congenital heart defects: An international retrospective cohort study. BMJ Open. 2019;9(7):e028139-028139.
3. Knowles RL, Bull C, Wren C, Dezateux C. Mortality with congenital heart defects in england and wales, 1959-2009: Exploring technological change through period and birth cohort analysis. Arch Dis Child. 2012;97(10):861-865.
4. Cuypers JA, Utens EM, Roos-Hesselink JW. Health in adults with congenital heart disease. Maturitas. 2016;91:69-73.
5. Dahlawi N, Milnes LJ, Swallow V. Behaviour and emotions of children and young people with congenital heart disease: A literature review. J Child Health Care. 2019:1367493519878550.
6. Hartman RJ, Rasmussen SA, Botto LD, et al. The contribution of chromosomal abnormalities to congenital heart defects: A population-based study. Pediatr Cardiol. 2011;32(8):1147-1157.
7. Vecoli C, Pulignani S, Foffa I, Andreassi MG. Congenital heart disease: The crossroads of genetics, epigenetics and environment. Curr Genomics. 2014;15(5):390-399.
8. Firth HV, Hurst JA. Oxford desk reference: Clinical genetics and genomics. Oxford: Oxford University Press, Incorporated; 2017. http://ebookcentral.proquest.com/lib/rug/detail.action?docID=5041403.
9. Baardman ME, Kerstjens-Frederikse WS, Corpeleijn E, et al. Combined adverse effects of maternal smoking and high body mass index on heart development in offspring: Evidence for interaction? Heart. 2012;98(6):474-479.
10. Zhao L, Chen L, Yang T, et al. Parental smoking and the risk of congenital heart defects in offspring: An updated meta-analysis of observational studies. Eur J Prev Cardiol. 2019:2047487319831367.
11. Feng Y, Wang S, Chen R, Tong X, Wu Z, Mo X. Maternal folic acid supplementation and the risk of congenital heart defects in offspring: A meta-analysis of epidemiological observational studies. Sci Rep. 2015;5:8506.
12. Gorini F, Chiappa E, Gargani L, Picano E. Potential effects of environmental chemical contamination in congenital heart disease. Pediatr Cardiol. 2014;35(4):559-568.
13. Zhang N, Chen M, Li J, et al. Metal nickel exposure increase the risk of congenital heart defects occurrence in offspring: A case-control study in china. Medicine (Baltimore). 2019;98(18):e15352.
14. Spinder N, Prins JR, Bergman JEH, et al. Congenital anomalies in the offspring of occupationally exposed mothers: A systematic review and meta-analysis of studies using expert assessment for occupational exposures. Hum Reprod. 2019;34:903-919
15. Eurocat Northern Netherlands. Registry description. https://www.umcg.nl/SiteCollectionDocuments/Zorg/Ouders/Eurocat/Registry_description_NNL_final_version_jan2018.pdf. Updated 2018. Accessed 1/21, 2020.
16. Scholtens S, Smidt N, Swertz MA, et al. Cohort profile: LifeLines, a three-generation cohort study and biobank. Int J Epidemiol. 2015;44(4):1172-1180.
17. EUROCAT. EUROCAT guide 1.4: Instruction for the registration of congenital anomalies. 2013.

18. Greenlees R, Neville A, Addor MC, et al. Paper 6: EUROCAT member registries: Organization and activities. Birth Defects Res A Clin Mol Teratol. 2011;91 Suppl 1:S51-S100.

19. Botto LD, Lin AE, Riehle-Colarusso T, Malik S, Correa A, National Birth Defects Prevention Study. Seeking causes: Classifying and evaluating congenital heart defects in etiologic studies. Birth Defects Res A Clin Mol Teratol. 2007;79(10):714-727.

20. Riehle-Colarusso T, Strickland MJ, Reller MD, et al. Improving the quality of surveillance data on congenital heart defects in the metropolitan atlanta congenital defects program. Birth Defects Res A Clin Mol Teratol. 2007;79(11):743-753.

21. International Labor Office. International standard classification of occupations. 1988.

22. Kromhout H, Vermeulen R. Application of job-exposure matrices in studies of the general population: Some clues to their performance. Eur Respir Rev. 2001(11):80-90.

23. Matheson MC, Benke G, Raven J, et al. Biological dust exposure in the workplace is a risk factor for chronic obstructive pulmonary disease. Thorax. 2005;60(8):645-651.

24. Bové H, Bongaerts E, Slenders E, et al. Ambient black carbon particles reach the fetal side of human placenta. Nature communications. 2019;10(1):3866.

25. Hansen JM. Oxidative stress as a mechanism of teratogenesis. Birth defects research.Part C, Embryo today : reviews. 2006;78(4):293-307.

26. Wang C, Zhan Y, Wang F, et al. Parental occupational exposures to endocrine disruptors and the risk of simple isolated congenital heart defects. Pediatr Cardiol. 2015;36(5):1024-1037.

27. Siegel M, Rocheleau CM, Johnson CY, et al. Maternal occupational oil mist exposure and birth defects, national birth defects prevention study, 1997-2011. International journal of environmental research and public health. 2019;16(9):1560.

28. Tikkanen J, Heinonen OP. Risk factors for coarctation of the aorta. Teratology. 1993;47(6):565-572.

29. Snijder CA, Vlot IJ, Burdorf A, et al. Congenital heart defects and parental occupational exposure to chemicals. Hum Reprod. 2012;27(5):1510-1517.

30. Cordier S, Bergeret A, Goujard J, et al. Congenital malformations and maternal occupational exposure to glycol ethers. Epidemiology. 1997;8(4):355-363.

31. Gilboa SM, Desrosiers TA, Lawson C, et al. Association between maternal occupational exposure to organic solvents and congenital heart defects, national birth defects prevention study, 1997-2002. Occup Environ Med. 2012;69(9):628-635.

32. Rocheleau CM, Bertke SJ, Lawson CC, et al. Maternal occupational pesticide exposure and risk of congenital heart defects in the national birth defects prevention study. Birth Defects Res Part A Clin Mol Teratol. 2015;103(10):823-833.

33. Teschke K, Olshan AF, Daniels JL, et al. Occupational exposure assessment in case-control studies: Opportunities for improvement. Occup Environ Med. 2002;59(9):575-93; discussion 594.

34. Banks E, Herbert N, Mather T, Rogers K, Jorm L. Characteristics of australian cohort study participants who do and do not take up an additional invitation to join a long-term biobank: The 45 and up study. BMC research notes. 2012;5:655.

35. Langhammer A, Krokstad S, Romundstad P, Heggland J, Holmen J. The HUNT study: Participation is associated with survival and depends on socioeconomic status, diseases and symptoms. BMC medical research methodology. 2012;12:143.

36. Klijs B, Scholtens S, Mandemakers JJ, Snieder H, Stolk RP, Smidt N. Representativeness of the LifeLines cohort study. PLoS One. 2015;10(9):e0137203.

37. 't Mannetje A, Kromhout H. The use of occupation and industry classifications in general population studies. Int J Epidemiol. 2003;32(3):419-428.

SUPPLEMENTARY FILES

Supplementary Table 1 | Prevalence, crude OR and adjusted OR of maternal occupational exposures and CHDs in offspring.

	Controls		CHDs		Unadjusted		Adjusted [a]	
	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Any CHD	(n=5602)		(n=1174)					
No exposure	5179	(92.4%)	1053	(89.7%)	Ref		Ref	
Mineral dust only	403	(7.2%)	109	(9.3%)	1.33	1.07-1.66	1.22	0.95-1.57
Metal dust and fumes only	5	(0.1%)	<5		NC		NC	
Both	15	(0.3%)	11	(0.9%)	**3.61**	**1.65-7.88**	**2.92**	**1.23-6.92**
Septal defects		(n=5602)		(n=544)				
No exposure	5179	(92.4%)	496	(91.2%)	Ref		Ref	
Mineral dust only	403	(7.2%)	42	(7.7%)	1.09	0.78-1.52	0.96	0.67-1.38
Metal dust and fumes only	13	(1.1%)	<5		NC		NC	
Both	15	(0.3%)	6	(1.1%)	**4.18**	**1.61-10.81**	**3.23**	**1.14-9.11**

CHD, congenital heart defects; LVOTO, left ventricular outflow tract obstruction; NC, not calculated due to sparse data. [a] Adjusted for child sex, maternal age at delivery (as continuous variable), educational level, maternal BMI (as continuous variable), smoking and alcohol use during pregnancy, folic acid supplementation, and fertility problems.

Supplementary Table 2 | Prevalence, crude OR and adjusted OR of maternal periconceptional exposure and isolated CHDs in the offspring.

	Occupational exposure																		
	Any exposure							Organic dust						Mineral dust					
	Total	Exposed		Unadjusted		Adjusted [a]		Exposed		Unadjusted		Adjusted [a]		Exposed		Unadjusted		Adjusted [a]	
CHD classification	n	n	%	OR	95% CI	OR	95% CI	n	%	OR	95% CI	OR	95% CI	n	%	OR	95% CI	OR	95% CI
Controls	5602	1992	(35.6%)	Ref		Ref	1992	1617	(28.9%)	Ref		Ref		418	(7.5%)	Ref		Ref	
Total CHD	1009	374	(37.1%)	1.07	0.93-1.23	1.02	0.871.19	300	(29.7%)	1.04	0.90-1.21	1.07	0.91-1.26	104	(10.3%)	**1.43**	**1.14-1.79**	**1.33**	**1.03-1.72**
Conotruncal	144	55	(38.2%)	1.12	0.80-1.57	1.08	0.75-1.55	45	(31.3%)	1.12	0.78-1.60	1.21	0.84-1.76	13	(9.0%)	1.23	0.69-2.20	1.18	0.64-2.18
d-TGA	64	23	(35.9%)	1.02	0.61-1.70	0.95	0.54-1.65	18	(28.1%)	0.96	0.56-1.67	1.06	0.60-1.89	<5		NC		NC	
Tetralogy of Fallot	45	21	(46.7%)	1.59	0.88-2.86	1.56	0.85-2.88	17	(37.8%)	1.50	0.82-2.74	1.60	0.85-2.96	5	(11.1%)	1.55	0.61-3.95	1.43	0.53-3.82
LVOTO	155	55	(35.5%)	1.00	0.72-1.39	0.92	0.64-1.32	46	(29.7%)	1.04	0.73-1.48	1.09	0.75-1.58	21	(13.5%)	**1.94**	**1.21-3.11***	**1.93**	**1.15-3.23**
HLHS	45	17	(37.8%)	1.10	0.60-2.02	0.83	0.44-1.59	13	(28.9%)	1.00	0.52-1.91	0.80	0.40-1.61	7	(15.6%)	**2.29**	**1.01-5.15**	1.82	0.74-4.51
Aortic stenosis	28	5	(17.9%)	0.40	0.15-1.04	0.37	0.13-1.10	<5		NC		NC		<5		NC		NC	
Coarctation of aorta	39	16	(41.0%)	1.26	0.66-2.39	1.19	0.61-2.34	15	(38.5%)	1.54	0.81-2.94	1.73	0.89-3.37	7	(17.9%)	**2.71**	**1.19-6.18**	**3.12**	**1.27-7.65**
Bicuspid aortic valve	37	15	(40.5%)	1.24	0.64-2.39	1.25	0.63-2.49	13	(35.1%)	1.34	0.68-2.63	1.49	0.74-2.99	5	(13.5%)	1.94	0.75-5.00	1.73	0.64-4.69
RVOTO	127	52	(40.9%)	1.26	0.88-1.80	1.16	0.80-1.68	43	(33.9%)	1.26	0.87-1.83	1.24	0.84-1.82	17	(13.4%)	**1.92**	**1.14-3.23**	**1.61**	**0.91-2.84**
P(v)S	94	56	(40.4%)	1.23	0.81-1.86	1.12	0.73-1.73	31	(33.0%)	1.21	0.79-1.87	1.18	0.75-1.84	14	(14.9%)	**2.17**	**1.22-3.86***	1.79	0.95-3.37
Pulmonary atresia	13	6	(46.2%)	1.55	0.52-4.63	1.37	0.45-4.20	6	(46.2%)	2.11	0.71-6.30	2.13	0.70-6.43	<5		NC		NC	
Septal	459	164	(35.7%)	1.01	0.83-1.23	0.99	0.80-1.22	129	(28.1%)	0.96	0.78-1.19	1.01	0.80-1.26	41	(8.9%)	1.22	0.87-1.70	1.12	0.78-1.62
Perimembranous VSD	91	41	(45.1%)	1.49	0.98-2.25	1.50	0.97-2.32	33	(36.3%)	1.40	0.91-2.16	1.54	0.99-2.40	10	(11.0%)	1.53	0.79-2.98	1.47	0.73-2.98
Muscular VSD	218	70	(32.1%)	0.86	0.64-1.15	0.88	0.65-1.20	57	(26.1%)	0.87	0.64-1.19	0.94	0.68-1.29	19	(8.7%)	1.18	0.73-1.92	1.23	0.74-2.06
Other VSD	67	22	(32.8%)	0.89	0.53-1.48	0.95	0.56-1.63	14	(20.9%)	0.65	0.36-1.18	0.76	0.41-1.40	5	(7.5%)	1.00	0.40-2.50	1.02	0.39-2.66
ASD	82	31	(37.8%)	1.10	0.70-1.73	0.97	0.60-1.55	25	(30.5%)	1.08	0.67-1.74	1.03	0.63-1.68	7	(8.5%)	1.16	0.53-2.53	0.88	0.38-2.00
AVSD	24	5	(20.8%)	0.48	0.18-1.28	0.51	0.18-1.42	<5		NC		NC		<5		NC		NC	
APVR	15	8	(53.3%)	2.07	0.75-5.72	1.83	0.63-5.34	7	(46.7%)	2.16	0.78-5.96	1.87	0.64-5.45	<5		NC		NC	
Total APVR	11	5	(45.5%)	1.51	0.46-4.96	1.48	0.44-4.97	5	(45.5%)	2.05	0.63-6.74	2.03	0.61-6.76	<5		NC		NC	
Complex	38	16	(42.1%)	1.32	0.69-2.52	1.18	0.58-2.37	13	(34.2%)	1.28	0.65-2.51	1.22	0.59-2.53	<5		NC		NC	
Single ventricle	11	7	(63.6%)	3.17	0.93-10.85	2.56	0.72-9.09	5	(45.5%)	2.05	0.63-6.74	1.96	0.58-6.64	<5		NC		NC	
Associations																			
CoA + VSD	14	7	(50.0%)	1.81	0.64-5.17	1.88	0.63-5.58	5	(35.7%)	1.37	0.46-4.09	1.50	0.49-4.59	<5		NC		NC	
P(v)S + VSD	17	9	(52.9%)	2.04	0.79-5.29	2.46	0.92-6.57	7	(41.2%)	1.73	0.66-4.54	2.07	0.77-5.56	<5		NC		NC	

Supplementary Table 2. Continued

	Occupational exposure																			
	Solvents							Pesticides						Metal dust and fumes						
	Total	Exposed		Unadjusted		Adjusted [a]		Exposed		Unadjusted		Adjusted [a]		Exposed		Unadjusted		Adjusted [a]	
CHD classification	n	n	%	OR	95% CI	OR	95% CI	n	%	OR	95% CI	OR	95% CI	n	%	OR	95% CI	OR	95% CI
Controls	5602	1370	(24.5%)	Ref		Ref		131	(2.3%)	Ref		Ref		20	(0.4%)	Ref		Ref	
Total CHD	1174	238	(23.6%)	0.95	0.82-1.12	0.95	0.80-1.13	29	(2.9%)	1.24	0.82-1.24	1.20	0.77-1.86	12	(1.2%)	**3.36**	**1.64-6.90***	**2.81**	**1.28-6.16**
Conotruncal	144	35	(24.3%)	0.99	0.68-1.46	1.07	0.72-1.58	<5		NC		NC		<5		NC		NC	
d-TGA	64	15	(23.4%)	0.95	0.53-1.67	1.10	0.60-1.99	<5		NC		NC		<5		NC		NC	
Tetralogy of Fallot	45	15	(33.3%)	1.55	0.83-2.88	1.55	0.82-2.93	<5		NC		NC		<5		NC		NC	
LVOTO	155	32	(20.6%)	0.80	0.54-1.19	0.77	0.51-1.17	7	(4.5%)	1.98	0.91-4.30	1.98	0.88-4.46	<5		NC		NC	
HLHS	45	9	(20.0%)	0.77	0.37-1.61	0.63	0.29-1.37	<5		NC		NC		<5		NC		NC	
Aortic stenosis	28	<5		NC		NC		<5		NC		NC		<5		NC		NC	
Coarctation of aorta	39	7	(17.9%)	0.68	0.30-1.53	0.69	0.30-1.58	<5		NC		NC		<5		NC		NC	
Bicuspid aortic valve	37	10	(27.0%)	1.14	0.55-2.37	1.25	0.59-2.65	<5		NC		NC		<5		NC		NC	
RVOTO	127	33	(26.0%)	1.08	0.73-1.62	1.08	0.72-1.63	<5		NC		NC		<5		NC		NC	
P(v)S	94	22	(23.4%)	0.94	0.58-1.53	0.95	0.58-1.55	<5		NC		NC		<5		NC		NC	
Pulmonary atresia	13	<5		NC		NC		<5		NC		NC		<5		NC		NC	
Septal	459	107	(23.3%)	0.94	0.75-1.18	0.96	0.75-1.21	14	(3.1%)	1.31	0.75-2.30	1.27	0.70-2.28	6	(1.3%)	**3.70**	**1.48-9.25***	**3.06**	**1.14-8.23**
Perimembranous VSD	91	27	(29.7%)	1.30	0.83-2.05	1.38	0.87-2.20	5	(5.5%)	2.3	0.97-6.08	2.33	0.91-6.00	<5		NC		NC	
Muscular VSD	218	48	(22.0%)	0.87	0.63-1.21	0.89	0.63-1.24	<5		NC		NC		<5		NC		NC	
Other VSD	67	12	(17.9%)	0.67	0.36-1.26	0.72	0.38-1.37	<5		NC		NC		<5		NC		NC	
ASD	82	20	(24.4%)	1.00	0.60-1.66	0.95	0.56-1.62	<5		NC		NC		<5		NC		NC	
AVSD	<5		NC		NC			<5		NC		NC		<5		NC		NC	
APVR	17	7	(46.7%)	2.70	0.98-7.47	2.07	0.71-6.03	<5		NC		NC		<5		NC		NC	
Total APVR	11	5	(45.5%)	2.57	0.78-8.45	2.23	0.67-7.42	<5		NC		NC		<5		NC		NC	
Complex	38	12	(31.6%)	1.43	0.72-2.83	1.41	0.68-2.92	<5		NC		NC		<5		NC		NC	
Single ventricle	11	<5		NC		NC		<5		NC		NC		<5		NC		NC	
Associations																			
CoA + VSD	14	<5		NC		NC		<5		NC		NC		<5		NC		NC	
P(v)S + VSD	17	6	(35.3%)	1.69	0.62-4.57	1.71	0.62-4.74	<5		NC		NC		<5		NC		NC	

CHD, congenital heart defects; d-TGA, dextro-transposition of the great arteries; LVOTO, left ventricular outflow tract obstruction; HLHS, hypoplastic left heart syndrome; RVOTO, right ventricular outflow tract obstruction; P(v)S, pulmonary (valve) stenosis; CoA, coarctation of aorta; VSD, ventricular septal defect; ASD, atrial septal defect; AVSD, atrioventricular septal defect; APVR, anomalous pulmonary venous return; NC, not calculated due to sparse data. [a] Adjusted for child sex, maternal age at delivery (as continuous variable), education level, maternal BMI (as continuous variable), smoking and alcohol use during pregnancy, folic acid supplementation, and fertility problems. *p-value <0.01.

Supplementary Table 3 | Prevalence, crude OR and adjusted OR of maternal periconceptional exposure and multiple CHDs in the offspring.

	Occupational exposure																		
	Any exposure							Organic dust						Mineral dust					
	Total	Exposed		Unadjusted		Adjusted [a]		Exposed		Unadjusted		Adjusted [a]		Exposed		Unadjusted		Adjusted [a]	
CHD classification	n	n	%	OR	95% CI	OR	95% CI	n	%	OR	95% CI	OR	95% CI	n	%	OR	95% CI	OR	95% CI
Controls	5602	1992	(35.6%)	Ref		Ref	1992	109	(66.1%)	Ref		Ref		418	(7.5%)	Ref		Ref	
Total CHD	165	68	(41.1%)	1.27	0.93-1.74	1.23	0.88-1.73	56	(33.9%)	1.27	0.91-1.76	1.37	0.97-1.94	16	(9.7%)	1.33	0.79-2.25	1.13	0.64-2.01
Conotruncal	30	14	(46.7%)	1.59	0.77-3.26	1.42	0.65-3.10	12	(40.0%)	1.64	0.79-3.42	1.78	0.81-3.89	5	(16.7%)	2.48	0.95-6.51	2.01	0.66-6.16
d-TGA	10	5	(50.0%)	1.81	0.52-6.27	1.43	0.37-5.54	5	(50.0%)	2.46	0.71-8.52	2.16	0.57-8.22	<5		NC		NC	
Tetralogy of Fallot	15	7	(46.7%)	1.59	0.57-4.38	1.32	0.45-3.92	6	(40.0%)	1.64	0.58-4.62	1.95	0.66-5.72	<5		NC		NC	
LVOTO	18	7	(38.9%)	1.15	0.45-2.98	1.09	0.41-2.89	7	(38.9%)	1.57	0.61-4.05	1.58	0.60-4.14	<5		NC		NC	
RVOTO	12	6	(50.0%)	1.81	0.58-5.63	2.32	0.73-7.40	6	(50.0%)	2.46	0.79-7.65	3.11	0.97-9.99	<5		NC		NC	
Septal	85	30	(35.3%)	0.99	0.63-1.55	0.88	0.54-1.42	21	(24.7%)	0.81	0.49-1.33	0.86	0.52-1.44	7	(8.2%)	1.11	0.51-2.43	0.85	0.37-1.94
Perimembranous VSD	26	10	(38.5%)	1.13	0.51-2.50	0.93	0.39-2.18	7	(26.9%)	0.91	0.38-2.16	1.04	0.43-2.56	<5		NC		NC	
Muscular VSD	30	9	(30.0%)	0.78	0.36-1.70	0.79	0.35-1.78	6	(20.0%)	0.62	0.25-1.51	0.70	0.28-1.76	<5		NC		NC	
Other VSD	11	5	(45.5%)	1.51	0.46-4.96	1.45	0.40-5.24	<5		NC		NC		<5		NC		NC	
ASD	16	5	(31.3%)	0.82	0.29-2.37	0.78	0.26-2.33	<5		NC		NC		<5		NC		NC	
AVSD	4	<5		NC		NC		<5		NC		NC		<5		NC		NC	
APVR	2	<5		NC		NC		<5		NC		NC		<5		NC		NC	
Complex	7	<5		NC		NC		<5		NC		NC		<5		NC		NC	

Supplementary Table 3. Continued

		Occupational exposure											
		Solvents						Pesticides					
	Total	Exposed		Unadjusted		Adjusted [a]		Exposed		Unadjusted		Adjusted [a]	
CHD classification	n	n	%	OR	95% CI	OR	95% CI	n	%	OR	95% CI	OR	95% CI
Controls	5602	1370	(24.5%)	Ref		Ref		131	(2.3%)	Ref		Ref	
Total CHD	165	37	(22.4%)	0.89	0.62-1.29	0.96	0.66-1.41	5	(3.0%)	1.31	0.53-3.23	1.27	0.50-3.24
Conotruncal	30	5	(16.7%)	0.62	0.24-1.62	0.70	0.26-1.86	<5		NC		NC	
d-TGA	10	<5		NC		NC		<5		NC		NC	
Tetralogy of Fallot	15	<5		NC		NC		<5		NC		NC	
LVOTO	18	5	(27.8%)	1.19	0.42-3.34	1.19	0.42-3.37	<5		NC		NC	
RVOTO	12	<5		NC		NC		<5		NC		NC	
Septal	85	14	(16.5%)	0.61	0.34-1.08	0.65	0.36-1.18	<5		NC		NC	
Perimembranous VSD	26	5	(19.2%)	0.74	0.28-1.95	0.79	0.29-2.16	<5		NC		NC	
Muscular VSD	30	<5		NC		NC		<5		NC		NC	
Other VSD	11	<5		NC		NC		<5		NC		NC	
ASD	16	<5		NC		NC		<5		NC		NC	
AVSD	4	<5		NC		NC		<5		NC		NC	
APVR	2	<5		NC		NC		<5		NC		NC	
Complex	7	<5		NC		NC		<5		NC		NC	

CHD, congenital heart defects; d-TGA, dextro-transposition of the great arteries; LVOTO, left ventricular outflow tract obstruction; HLHS, hypoplastic left heart syndrome; RVOTO, right ventricular outflow tract obstruction; P(v)S, pulmonary (valve) stenosis; CoA, coarctation of aorta; VSD, ventricular septal defect; ASD, atrial septal defect; AVSD, atrioventricular septal defect; APVR, anomalous pulmonary venous return; NC, not calculated due to sparse data. [a] Adjusted for child sex, maternal age at delivery (as continuous variable), education level, maternal BMI (as continuous variable), smoking and alcohol use during pregnancy, folic acid supplementation, and fertility problems. *p-value <0.01. Exposure to metal dust and fumes was not calculated due to zero exposed cases.

Supplementary Table 4 | Prevalence, crude OR and adjusted OR of maternal occupational exposure and CHDs in the offspring.

	Controls (n=5602)		CHDs (n=1174)		Unadjusted		Adjusted [a]	
	n	(%)	n	(%)	OR	95% CI	OR	95% CI
Any exposure								
No exposure	3611	(64.5%)	733	(62.4%)	Ref		Ref	
Low exposure	1802	(32.2%)	386	(32.9%)	1.06	0.92-1.21	1.00	0.86-1.16
High exposure	189	(3.4%)	55	(4.7%)	**1.43**	**1.05-1.96**	1.37	0.97-1.94

CHD, congenital heart defects. [a] Adjusted for child sex, maternal age at delivery (as continuous variable), educational level, maternal BMI (as continuous variable), smoking and alcohol use during pregnancy, folic acid supplementation, and fertility problems.

CHAPTER 6

Maternal occupational exposure to solvents and gastroschisis in offspring, National Birth Defects Prevention Study 1997-2011

Nynke Spinder
Lynn M. Almli
Tania A. Desrosiers
Kathryn E. Arnold
Jorieke E.H. Bergman
Hans Kromhout
H. Marike Boezen
Hermien E.K de Walle
Carissa M. Rocheleau
Jennita Reefhuis
and the National Birth Defects Prevention Study

Occupational and Environmental Medicine. 2020;77:172-178

ABSTRACT

Objectives The aim of this study was to assess the association between maternal occupational exposure to solvents and gastroschisis in offspring.

Methods We used data from the National Birth Defects Prevention Study, a large population-based case-control study of major birth defects conducted in ten U.S. states from 1997-2011. Infants with gastroschisis were ascertained by active birth defects surveillance systems. Control infants without major birth defects were selected from vital records or birth hospital records. Self-reported maternal occupational histories were collected by telephone interview. Industrial hygienists reviewed this information to estimate exposure to aromatic, chlorinated, and petroleum-based solvents from one month before conception through the first trimester of pregnancy. Cumulative exposure to solvents was estimated for the same period accounting for estimated exposure intensity and frequency, job duration, and hours worked per week. Odds ratios (ORs) and 95% confidence intervals (95%CIs) were estimated to assess the association between exposure to any solvents or solvent classes, and gastroschisis risk.

Results Among 879 cases and 7817 controls, the overall prevalence of periconceptional solvent exposure was 7.3% and 7.4% respectively. Exposure to any solvent versus no exposure to solvents was not associated with gastroschisis after adjusting for maternal age (OR 1.00, 95%CI 0.75–1.32), nor was an association noted for solvent classes. There was no exposure-response relationship between estimated cumulative solvent exposure and gastroschisis after adjusting for maternal age.

Conclusion Our study found no association between maternal occupational solvent exposure and gastroschisis in offspring. Further research is needed to understand risk factors for gastroschisis.

INTRODUCTION

Gastroschisis is a severe birth defect of the abdominal wall, which involves a full-thickness para-umbilical defect through which intestines and other organs may herniate without a covering membrane. Gastroschisis is most often an isolated defect and is not associated with chromosomal disorders.[1] The prevalence of gastroschisis in the United States is increasing, and is currently estimated to be approximately 4.5 per 10,000 births.[2] The majority of infants need surgery to close the abdominal wall. After surgery, 90% of these infants are alive at one year of age.[3]

The aetiology of gastroschisis is unknown and much debated. One recent hypothesis is that gastroschisis develops due to rupture or non-closure of the membrane covering the umbilical ring between 8 and 11 weeks after fertilization;[4,5] however, other hypotheses are suggested.[6,7] The increased prevalence of gastroschisis suggests a role of unknown environmental factors, which might have an effect on the developing membrane of the umbilical ring.[8] Epidemiological studies show that the strongest risk factor for gastroschisis is young maternal age (<20 years of age).[9] Other risk factors associated with gastroschisis are maternal smoking,[9] alcohol consumption, illicit drugs,[10,11] and low maternal body mass index (BMI).[12] Maternal illnesses such as depression, urinary tract infections, and sexually transmitted diseases before or early in pregnancy;[8,13-15] and use of specific medications early in pregnancy [9,13,16] have also been associated with gastroschisis. The relationships between these risk factors and gastroschisis are complicated by young maternal age, since it is not clear whether maternal age is a confounder or on causal pathways involving these exposures and gastroschisis.

Fewer studies have examined the role of occupational exposures that might be associated with gastroschisis. Millions of workers in the United States are exposed to solvents, which are present in paints, adhesives, glues, and degreasing/cleaning agents. Solvents are used for production of plastics, textiles, printing inks, agricultural products, and pharmaceuticals.[17] Solvents are known for their reproductive toxicity,[18] and might therefore have an effect on the development of gastroschisis. A recent meta-analysis found that maternal occupational exposure to solvents before and during pregnancy is associated with several birth defects, including neural tube defects, congenital heart defects, and oral facial clefts.[19] One case-control study was conducted assessing maternal occupational exposure to solvents and gastroschisis. This study, including 110 gastroschisis cases and 220 controls, reported an association (odds ratio (OR) 2.55, 95% confidence interval (CI) 1.10-5.89).[20]

The aim of our study was to assess the association between estimated maternal occupational exposure to solvents during the periconceptional period (one month before conception through three months after conception) and gastroschisis in offspring using data from the National Birth Defects Prevention Study (NBDPS).

METHODS

Study design

The NBDPS is a large population-based multicenter case–control study of major structural birth defects in the United States. Detailed information about NBDPS has been previously described.[21] In brief, pregnancies with estimated delivery dates between October 1, 1997 and December 31, 2011 in Arkansas, California, Georgia, Iowa, Massachusetts, North Carolina, New Jersey, New York, Texas, and Utah were included.

All states included live-born cases, whereas most states also included cases among stillbirths (death after >20 of gestational age) and terminated pregnancies with a prenatal diagnosis of birth defects. Cases were ascertained by the participating states' birth defects surveillance systems up to 2 years after delivery. To confirm eligibility, clinical information abstracted from medical records was reviewed by a clinical geneticist at each center using a systematic study-wide classification protocol. Only one infant per family was eligible for the study. Controls were live-born infants without major birth defects selected randomly from either vital records or birth hospital records from the same geographical regions and time-period as cases. All participants gave informed consent.

Case classification

Cases were classified as "isolated" if they had one major defect or two major defects involving the same organ system; cases were classified as "multiple" if they had multiple major defects in different organ systems.[22] Infants were excluded if defects were related to a single gene condition or a chromosomal abnormality, or if case information was classified as limb-body wall complex or amniotic band sequence. Furthermore, infants with a first-degree family member with gastroschisis were excluded because of unknown heredity.

Exposure assessment

Women who participated in the NBDPS completed a computer assisted telephone interview (CATI) in English or Spanish between 6 weeks and 24 months after the estimated

delivery date. Mothers were asked to report information about demographics, medication use, and lifestyle during pregnancy and the three months preceding pregnancy. Occupational histories were collected among women who reported a job for at least one month or more during the three months prior to conception through the end of pregnancy. Women were asked about their job title, employer name, what the company makes or does, their primary tasks and duties, description of chemicals and machines handled on the job, dates of employment, and hours and days worked per week for each job.

All jobs were coded using the Standard Occupational Classification (SOC) 2010.[23] Industrial hygienists and occupational experts working at the National Institute for Occupational Safety and Health (NIOSH) performed, blinded by case-control status, a retrospective exposure assessment for a variety of occupational exposures, including ten solvents: benzene, xylene, toluene, carbon tetrachloride, chloroform, methylene chloride, perchloroethylene, 1,1,1-trichloroethane, trichloroethylene, and Stoddard solvent. Each job was assigned scores for estimated relative intensity of exposure (Supplementary Table 1) and frequency (none, >0 to <2 hours per week, 2-10 hours per week, 11-19 hours per week, >19 hours per week exposed in a standard 40-hour week), as well as probability and confidence scores to reflect the certainty of the raters. Probability score was defined as the estimated percentage of mothers with similar jobs being exposed to solvents (<10% – >90%). Confidence score was defined as the confidence of the industrial hygienist that mothers' job matched the job description indicating solvent exposure (low – very high). Raters compiled previously published exposure measurements from a variety of studies and workplace evaluations to guide them as they assigned ratings. If ratings between the hygienists disagreed, they met with an additional industrial hygienist/occupational health expert to discuss and reach consensus on the most appropriate rating.

To combine information on intensity and frequency of exposure, as well as self-reported hours worked per week and duration of the job during the window of biological interest, intensity and frequency scores were quantitatively mapped to the midpoint of their estimated range and calculated as follows: (intensity)×(frequency as hours per week/40 hours per week)×((self-reported work frequency (hours/week))/(7 days/week))×(number of days worked during the periconceptional period). This resulted in an estimated cumulative exposure (in parts per million (ppm)-hours or $\mu g/m^3$) for each job during the periconceptional period;[24] a similar approach has been described and used elsewhere.[25]

Although most mothers held one job, some mothers held multiple jobs during the periconceptional period. Mothers with multiple jobs were considered as exposed if any of her jobs during the periconceptional period was rated as exposed. If all jobs were rated as unexposed, mothers were considered to have been unexposed. The estimated cumulative exposure (ppm-hours or μg/m^3) was summed across all jobs. Mothers who reported not being employed during the periconceptional period were excluded from this analysis to reduce the potential for bias due to work status or employment-related factors.[26]

Statistical analysis

Frequency distributions of maternal demographic and behavioural characteristics were calculated for cases and controls. Additionally, frequency distributions for solvent-exposed and solvent-unexposed controls were calculated to give an overview of characteristics for the working population. The prevalence of 23 SOC major job groups for solvent-exposed and solvent-unexposed case and control mothers was tabulated to characterize the occupation types held in our exposed study population.

Correlations between exposure status within and between solvent classes were explored in mothers of controls to determine the best modelling strategy. Solvents were evaluated individually and subsequently grouped by class into aromatic solvents (benzene, xylene, toluene) and chlorinated solvents (carbon tetrachloride, chloroform, methylene chloride, perchloroethylene, 1,1,1-trichloroethane, trichloroethylene) due to high correlation within these groupings. For example, 98% of mothers exposed to trichloroethylene were also considered to be exposed to methylene chloride (n=259). Correlation between assigned solvent classes was substantially lower compared to correlation between individual chemicals within solvent classes (Supplementary Table 2).

The prevalence of occupational exposure (no exposure/exposure) was estimated for any solvent exposure and solvent classes (aromatic, chlorinated, and Stoddard solvents). Univariate logistic regression analyses were performed to estimate ORs and 95% CIs in order to assess the association between maternal occupational exposure to solvents and gastroschisis, using non-exposed mothers for the solvent class under analysis as the reference category. Sparse data (≤3 exposed individuals) were not presented, and ORs were not estimated. To assess covariates associated with gastroschisis and/or solvent exposure for the multivariate regression analyses, we introduced one covariate at a time into the model. At least a 10%-point difference in the OR for the main effect between solvents and gastroschisis was considered as a meaningful difference. We examined the following self-

reported covariates: NBDPS center, maternal education (≤12 and >12 years), race/ethnicity (Non-Hispanic white, Non-Hispanic black, Hispanic, and other), BMI (continuous), parity (0 and ≥1), maternal cigarette smoking including second-hand smoke at work or at home (yes/no), alcohol intake (yes/no), illicit drug use (yes/no) during the periconceptional period. None of these covariates produced a 10%-point difference in the OR for the main effect. Maternal age was *a priori* selected as covariate, due to the strong association between young maternal age and gastroschisis.

Sensitivity analyses were conducted in order to account for exposure misclassifications. First we repeated analyses restricting the exposed group to women with at least one job with an estimated probability of exposure ≥10%. Second, we repeated the solvent-gastroschisis analyses restricting to women with at least one job with medium/high confidence. Mothers with multiple jobs that changed exposure category due to those restrictions were excluded from analyses.

Because young maternal age is the strongest risk factor for gastroschisis, analyses stratified by maternal age (<20 and ≥20 years) were conducted. Furthermore, stratified analyses were conducted for isolated and multiple defects, since isolated and non-isolated defects may differ in aetiology.

Exposure-response analyses for overall solvent exposure and each solvent class were conducted to assess cumulative maternal occupational solvent exposure and gastroschisis. The estimated cumulative exposure was analysed in four groups, based on tertiles of the exposed controls (none, level 1, 2, and 3). Crude and adjusted ORs (aORs) and 95% CIs were estimated for the association between cumulative exposure to any solvents and classes and gastroschisis. Logistic regression was used to test for a linear trend in the betas of the tertiles of cumulative solvent exposure using the Wald test of significance. Separate analyses were conducted for intensity and frequency of exposure.

RESULTS

In total, 13,279 control infants or infants with gastroschisis were identified. One infant with an amniotic band sequence/limb-body-wall complex was excluded. There were 4,573 mothers excluded because no job was reported during the periconceptional period. They were homemakers (n=2,838), students (n=617), disabled (n=45), in between jobs (n=182), not specified (n=35), or were missing information about employment (n=485). Finally, 369 mothers were excluded because their reported job was not held during the

periconceptional period or because exposure could not by assigned (n=2). Four cases and five controls were excluded because they had a first-degree relative with gastroschisis. In total, 879 infants with gastroschisis and 7,817 control infants were included in this study.

The comparisons of maternal characteristic between cases and controls are shown in Table 1. Mothers of cases with gastroschisis were younger, had fewer years of education, were more likely to be Hispanic, and had a lower BMI. Mothers of cases had greater exposure to cigarette smoking, and used alcohol and illicit drugs more frequently during the periconceptional period compared to mothers of controls. Exposed mothers had significantly fewer years of education, had greater exposure to cigarette smoking, but consumed less alcohol than non-exposed mothers (Supplementary Table 3). Among cases, 96.2% were live births, 3.1% were foetal deaths (>20 weeks of gestational age), and 0.7% were induced abortions.

Table 1 | Baseline characteristics of gastroschisis cases and controls, National Birth Defects Prevention Study, USA, 1997-2011

	Gastroschisis cases (n = 879)		**Total Controls (n = 7817)**	
	N	(%)	N	(%)
Maternal age at delivery (years) †				
<20	246	(28.0%)	492	(6.3%)
20-24	408	(46.4%)	1747	(22.3%)
25-29	161	(18.3%)	2240	(28.7%)
30-34	52	(5.9%)	2163	(27.7%)
≥35	12	(1.4%)	1176	(15.0%)
Maternal education †				
≤12 years	498	(56.9%)	2504	(32.1%)
>12 years	377	(43.1%)	5300	(67.9%)
Maternal race-ethnicity †				
Non-Hispanic white	503	(57.2%)	5003	(64.0%)
Non-Hispanic black	83	(9.4%)	899	(11.5%)
Hispanic	223	(25.4%)	1433	(18.3%)
Other	70	(8.0%)	482	(6.2%)
Pre-pregnancy BMI (kg/m^2) †				
Underweight (<18.5)	63	(7.3%)	352	(4.6%)
Normal weight (18.5-25)	603	(69.6%)	4125	(53.9%)
Overweight (25-30)	159	(18.3%)	1772	(23.2%)
Obese (>30)	42	(4.8%)	1403	(18.3%)

Table 1. Continued

	Gastroschisis cases (n = 879)		Total Controls (n = 7817)	
	N	(%)	N	(%)
Parity †				
0	611	(69.5%)	3514	(45.0%)
≥1	268	(30.5%)	4301	(55.0%)
Maternal cigarette smoking during periconceptional period[a] †				
Yes	485	(55.2%)	2498	(32.0%)
No	391	(44.8%)	5302	(68.0%)
Maternal alcohol use during periconceptional period †				
Yes	429	(48.9%)	3327	(42.7%)
No	448	(51.2%)	4464	(56.7%)
Maternal illicit drug use during periconceptional period[b] †				
Yes	117	(13.3%)	329	(4.2%)
No	761	(86.7%)	7486	(95.8%)

Totals do not add up due to missing data. BMI = body mass index. [a] = self-reported cigarette smoking and second-hand cigarette smoke exposure at work and at home, [b] = included marijuana, hash, cocaine, crack, hallucinogens, heroin, and mushrooms, † = significant difference between cases and controls (p-value <0.05) using χ^2 tests.

The prevalence of estimated occupational exposure to any solvent during the periconceptional period was 7.3% among cases and 7.4% among controls (Table 2). Mothers with exposure to any solvents worked in production occupations (28.0%), personal care and service occupations (18.4%), building and grounds cleaning and maintenance occupations (12.9%). There was no association between maternal occupational exposure to solvents and gastroschisis (aOR 1.00, 95%CI 0.75-1.32, adjusted for maternal age) (Table 2). Exposure prevalence for aromatic solvents was 2.2% for cases and 2.1% for controls, and there was no association between aromatic solvents and gastroschisis (aOR 1.15, 95%CI 0.69-1.92). Exposure to chlorinated solvents was most common; 6.4% for both cases and controls. However, no increased OR was identified in association with gastroschisis (aOR 0.98, 95%CI 0.73-1.32). The prevalence of Stoddard solvents exposure was 2.2% for cases and 2.0% for controls, but no association between Stoddard solvents exposure and gastroschisis was found (aOR 0.84, 95%CI 0.51-1.39). When analyses were restricted to jobs with an estimated exposure probability ≥10%, similar results were observed compared to analyses that included all women (data not shown). In addition, analyses restricted to jobs with medium and high confidence of solvent exposure also showed similar results (data not shown).

Table 2 | Prevalence of estimated maternal occupational exposure to solvents during the periconceptional period[a] and risk of gastroschisis in offspring, National Birth Defects Prevention Study, USA, 1997-2011

Solvent classes	Gastroschisis cases (n = 879)		Total Controls (n = 7817)		Unadjusted		Adjusted[b]	
	N	(%)	N	(%)	OR	95% CI	OR	95% CI
Any solvent								
No exposure	813	(92.7%)	7233	(92.6%)	Ref		Ref	
Exposure	64	(7.3%)	579	(7.4%)	0.98	0.75 - 1.29	1.00	0.75 - 1.32
Aromatic solvents								
No exposure[c]	859	(97.8%)	7651	(97.9%)	Ref		Ref	
Exposure	19	(2.2%)	163	(2.1%)	1.04	0.64 - 1.68	1.15	0.69 - 1.92
Chlorinated solvents								
No exposure[c]	821	(93.6%)	7311	(93.6%)				
Exposure	56	(6.4%)	502	(6.4%)	0.98	0.75 - 1.32	0.98	0.73 - 1.32
Stoddard solvents								
No exposure[c]	858	(97.8%)	7658	(98.0%)	Ref		Ref	
Exposure	19	(2.2%)	158	(2.0%)	1.07	0.66 - 1.74	0.84	0.51 - 1.39

Totals do not add up due to missing data. [a] = one month before conception through three months after conception, [b] = adjusted for maternal age at delivery as a continuous variable (no missing values), [c] = no exposure for outcome under analysis.

Analysis stratified by maternal age at delivery (<20 and ≥20 years of age) showed that exposure to solvents was more prevalent among cases with older mothers (8.7%) compared to cases with younger mothers (3.7%) (data not shown). The OR for any solvent exposure versus no solvent exposure for older mothers showed no significant increase (OR 1.16, 95%CI 0.87-1.55), nor were increased ORs observed for solvent classes. The OR for any solvents among younger mothers showed no increase (OR 0.74, 95%CI 0.34-1.61). No increased ORs were found for solvents by class.

Stratified analysis by isolated and multiple defects included 801 cases with an isolated defect and 78 cases with multiple defects (Table 3). Exposure to any solvent was more common among exposed cases with multiple defects (14.1%) compared to exposed cases with isolated defects (6.6%). An increased OR was found for any solvent exposure (aOR 2.11, 95%CI 1.10-4.06) for infants with multiple defects. The estimate was lower for chlorinated solvents (aOR 1.44, 95%CI 0.65-3.17). The ORs for aromatic and Stoddard solvents could not be calculated due to sparse data (n ≤ 3). Increased ORs were not observed for isolated defects (e.g. any solvent exposure versus no solvent: aOR 0.90, 95%CI 0.66-1.21).

Table 3 | Prevalence of estimated maternal occupational exposure to solvents during the periconceptional period[a] and risk of gastroschisis in offspring, stratified by isolated versus multiple defects, National Birth Defects Prevention Study, USA, 1997-2011

	Isolated defects								**Multiple defects**							
	Exposed				Crude		Adjusted[b]		Exposed				Crude		Adjusted[b]	
Solvent classes	Cases (n=801)		Controls (n=7817)		OR	95% CI	aOR	95% CI	Cases (n=78)		Controls (n=7817)		OR	95% CI	aOR	95% CI
Any solvent	53	(6.6%)	579	(7.4%)	0.89	0.66 – 1.19	0.90	0.66 – 1.22	11	(14.1%)	579	(7.4%)	2.05	1.08 – 3.90	2.11	1.10 – 4.06
Aromatic solvents	16	(2.0%)	163	(2.1%)	0.96	0.57 – 1.61	1.05	0.61 – 1.81	<3				NC		NC	
Chlorinated solvent	49	(6.1%)	502	(6.4%)	0.95	0.70 – 1.29	0.94	0.69 – 1.29	7	(9.0%)	502	(6.4%)	1.44	0.66 – 3.14	1.44	0.65 – 3.17
Stoddard solvents	18	(2.3%)	158	(2.0%)	1.12	0.68 – 1.83	0.87	0.52 – 1.46	≤3				NC		NC	

NC = not calculated due to sparse data (n ≤ 3 individuals). [a] = one month before conception through three months after conception, [b] = adjusted for maternal age at delivery as a continuous variable (no missing values).

The prevalence and ORs for the estimated maternal cumulative exposure to solvents during the periconceptional period and gastroschisis in offspring are shown in Table 4. We did not observe an exposure level-response association for any solvent exposure, nor for aromatic, chlorinated, or Stoddard solvents exposure. No trends were observed for increasing cumulative maternal occupational exposures to solvents or to solvent classes. Exposure-response analyses could not be performed for multiple defects, due to too few cases per category. Separate analyses for intensity and frequency of exposure showed no differences between lower and higher intensities or frequencies of exposure (Supplementary Table 4 and 5).

Table 4 | Prevalence of cumulative maternal occupational exposure to solvents during the periconceptional period[a] and risk of gastroschisis in offspring, National Birth Defects Prevention Study, USA, 1997-2011

	Cases (n=879)[b]		**Controls (n = 7817)[b]**		**Unadjusted**		**Adjusted[c]**	
Solvent classes [d]	N	%	N	%	OR	95% CI	OR	95% CI
Any solvents					P_{trend} = 0.68		P_{trend}=0.79	
No exposure [e]	813	(92.7%)	7233	(92.6%)	Ref		Ref	
Level 1	14	(1.6%)	193	(2.5%)	0.64	0.37 – 1.11	0.68	0.38 – 1.20
Level 2	27	(3.1%)	191	(2.4%)	1.26	0.83 – 1.89	1.37	0.89 – 2.12
Level 3	23	(2.6%)	194	(2.5%)	1.05	0.68 – 1.63	0.96	0.60 – 1.51
Aromatic solvents					P_{trend} = 0.69		P_{trend} = 0.66	
No exposure [e]	859	(97.8%)	7651	(97.9%)	Ref		Ref	
Level 1	5	(0.6%)	54	(0.7%)	0.83	0.33 – 2.07	1.08	0.41 – 2.84
Level 2	7	(0.8%)	54	(0.7%)	1.16	0.52 – 2.55	1.50	0.64 – 3.52
Level 3	7	(0.8%)	54	(0.7%)	1.16	0.52 – 2.55	0.98	0.43 – 2.24
Chlorinated solvents					P_{trend} = 0.58		P_{trend} = 082	
No exposure[e]	821	(93.6%)	7311	(93.6%)	Ref		Ref	
Level 1	11	(1.3%)	167	(2.1%)	0.59	0.32 – 1.08	0.61	0.32 – 1.15
Level 2	24	(2.7%)	167	(2.1%)	1.28	0.83 – 1.98	1.41	0.89 – 2.24
Level 3	21	(2.4%)	167	(2.1%)	1.12	0.71 – 1.77	0.97	0.59 – 1.54
Stoddard solvents					P_{trend} = 0.95		P_{trend} = 0.37	
No exposure[e]	858	(97.8%)	7658	(98.0%)	Ref		Ref	
Level 1	7	(0.8%)	51	(0.7%)	1.23	0.55 – 2.71	0.93	0.41 – 2.14
Level 2	8	(0.9%)	54	(0.7%)	1.32	0.63 – 2.79	1.09	0.50 – 2.38
Level 3	4	(0.5%)	53	(0.7%)	0.67	0.24 – 1.87	0.52	0.18 – 1.48

[a] = one month before conception through three months after conception, [b] = missing cases/controls varied from 4 to 7 mothers across exposures because exposure could not be assigned or cumulative exposure could not be calculated, [c] = adjusted for maternal age at delivery as a continuous variable (no missing values), [d] = based on tertiles of the exposed controls. [e] = no exposure for outcome under analysis. P_{trend} = Wald p-value for testing linear trend of the tertile betas .

DISCUSSION

In this study we did not find an association between maternal occupational exposure to chlorinated, aromatic, or Stoddard solvents during the periconceptional period and isolated gastroschisis in offspring. We did observe an association between exposure to any solvents and gastroschisis co-occurring with other defects, but this should be interpreted with caution. The observed association did not reach statistical significance for aromatic and chlorinated solvents, but these analyses were based on a small number of multiple cases. Overall, the power of these analyses is limited; only 78 cases were included, of which 11 cases were exposed. Furthermore, gastroschisis is mainly known as an isolated defect. When we further explored the types of multiple defects in our study population, we did not identify a specific pattern among the defects in association with the gastroschisis. Most cases had one additional birth defect, such as a congenital heart defect or a neural tube defect, which have been previously associated with occupational solvent exposure.[19]

Stratification by maternal age showed no association between occupational exposure to solvents and gastroschisis. No exposure-response relationship for any solvents or solvent classes and gastroschisis were found.

One previous study reported an association between maternal occupational exposure to solvents and gastroschisis (OR 2.55, 95%CI 1.10-5.89).[20] This case-control study was performed by the California Birth Defects Monitoring Program in 1989 and 1990. Case/control ascertainment and inclusion criteria were comparable to the NBDPS. In this study by Torfs and colleagues, during an interview mothers were asked to describe any occupations performed, including specific tasks, during the three months before conception and the first trimester. One industrial hygienist, blinded by outcome, evaluated the type of exposure that was associated with the job. Solvent types included aromatic hydrocarbons, gaseous aliphatic hydrocarbons, and liquid aliphatic hydrocarbons. Exposure assessment was comparable to our exposure assessment. However, we used a multiple expert rater method of exposure assessment, which is known to reduce exposure misclassification.[27] The prevalence of exposure was not reported for occupational exposure specifically, and could therefore not be compared to our exposure prevalence. Finally, we included 879 cases with gastroschisis whereas Torfs and colleagues included only 150 cases. Their study did not report on whether cases had isolated defects or multiple defects including gastroschisis. Therefore, our results regarding multiple defects could not be compared. In

conclusion, differences in results could be explained by different inclusion criteria, possible exposure misclassification, and a difference in power.

Strengths and limitations

One strength of this study is that we utilized data from the NBDPS, a large population-based case-control study in which ten centers participated for most of the study period. Each center covered a birth population between 35,000 and 80,000 births per year.[21] Therefore, a relatively large number of infants with gastroschisis could be included. Live births, stillbirths, and terminated pregnancies were included in most states, thereby mitigating selection bias due to survival. In addition, careful clinical review and classification by clinical geneticists were conducted, reducing outcome misclassification. Finally, the NBDPS included control infants without major birth defects. These infants were generally representative of the base population from which they were selected[28].

Another strength of this study is that we restricted our study sample to women who reported having a job during the periconceptional period. This is important because employment status is related to sociodemographic and (reproductive) health characteristics that are generally recognized risk factors for adverse pregnancy outcomes. By restricting our analyses to employed women, we controlled for confounding by employment status and related factors.[26,29] The interrater reliability of exposure assessment used in this study was fair to good and was generally comparable to or slightly higher than reliability estimates from similar studies, therefore it might be less likely that exposure misclassification impacted our results.[24]

Despite our large study sample, the number of exposed cases was relatively low (7%) compared to other population-based studies of occupational solvent exposure during pregnancy (10-19%[19]) using similar exposure assessment methods. This could have resulted in imprecision of our estimates. This is especially true for the exposure-response analyses where less than 3% of exposed cases per level were included. With three levels of exposure, we created a contrast between low and high exposure; however, this resulted in lower power compared to the analysis with two exposure categories. Our estimates were generally more precise than the previous study[20], likely due to the unprecedented number of cases available in NBDPS. However, direct comparison to previous work is tenuous given the differences in exposure assessment methodologies. Most women in this population-based study were exposed to relatively low estimated doses of solvents. However, we cannot rule out effects among workers with much higher doses of exposures.

A limitation of exposure assessment is that non-differential misclassification of exposure could have occurred, because assessment was indirect and retrospective. We possibly reduced potential misclassification by looking only at solvent class and not at individual solvents. The sensitivity and specificity of exposure assessment by industrial hygienist is unknown compared with true exposure, since there was no validation by direct exposure measurement. Another limitation of retrospective exposure assessment is the possibility that women avoided or were restricted by their employer to handle certain solvents during work, or wore protective equipment because they wanted to become pregnant or knew they were pregnant.

A limitation of the NBDPS is that selection bias could have occurred, since approximately two-thirds of invited women participated (65% for cases and controls).[21] However, a previous study showed that NBDPS participants held a wide variety of occupations.[30]

Conclusion

We did not observe an association between gastroschisis in offspring and estimated maternal occupational exposure to solvents and solvent classes during the periconceptional period in this large population-based case-control study. Among mothers with gastroschisis cases with multiple defects, an association with maternal occupational exposure to solvents was observed, but these results should be interpreted with caution. No exposure-response relationship was observed using estimated cumulative occupational exposure to solvents. Continued exploration of risk factors for gastroschisis is warranted.

REFERENCES

1. Prefumo F, Izzi C. Fetal abdominal wall defects. Best Pract Res Clin Obstet Gynaecol. 2014;28(3):391-402.
2. Short TD, Stallings EB, Isenburg J, et al. Gastroschisis trends and ecologic link to opioid prescription rates - united states, 2006-2015. MMWR Morb Mortal Wkly Rep. 2019;68(2):31-36.
3. Fillingham A, Rankin J. Prevalence, prenatal diagnosis and survival of gastroschisis. Prenat Diagn. 2008;28(13):1232-1237.
4. Bargy F, Beaudoin S. Comprehensive developmental mechanisms in gastroschisis. Fetal Diagn Ther. 2014;36(3):223-230.
5. Opitz JM, Feldkamp ML, Botto LD. An evolutionary and developmental biology approach to gastroschisis. Birth Defects Res. 2019;111(6):294-311.
6. Feldkamp ML, Carey JC, Sadler TW. Development of gastroschisis: Review of hypotheses, a novel hypothesis, and implications for research. Am J Med Genet A. 2007;143A(7):639-652.
7. Lubinsky M. A vascular and thrombotic model of gastroschisis. Am J Med Genet A. 2014;164A(4):915-917.
8. Feldkamp ML, Arnold KE, Krikov S, et al. Risk of gastroschisis with maternal genitourinary infections: The US national birth defects prevention study 1997-2011. BMJ Open. 2019;9(3):e026297-026297.
9. Mac Bird T, Robbins JM, Druschel C, Cleves MA, Yang S, Hobbs CA. Demographic and environmental risk factors for gastroschisis and omphalocele in the national birth defects prevention study. Journal of Pediatric Surgery. 2009;44(8):1546-1551.
10. van Gelder MM, Donders AR, Devine O, Roeleveld N, Reefhuis J, National Birth Defects Prevention Study. Using bayesian models to assess the effects of under-reporting of cannabis use on the association with birth defects, national birth defects prevention study, 1997-2005. Paediatr Perinat Epidemiol. 2014;28(5):424-433.
11. Draper ES, Rankin J, Tonks AM, et al. Recreational drug use: A major risk factor for gastroschisis? Am J Epidemiol. 2008;167(4):485-491.
12. Waller DK, Shaw GM, Rasmussen SA, et al. Prepregnancy obesity as a risk factor for structural birth defects. Arch Pediatr Adolesc Med. 2007;161(8):745-750.
13. Given JE, Loane M, Garne E, et al. Gastroschisis in europe - A case-malformed-control study of medication and maternal illness during pregnancy as risk factors. Paediatr Perinat Epidemiol. 2017;31(6):549-559.
14. Feldkamp ML, Reefhuis J, Kucik J, et al. Case-control study of self reported genitourinary infections and risk of gastroschisis: Findings from the national birth defects prevention study, 1997-2003. BMJ. 2008;336(7658):1420-1423.
15. Yazdy MM, Mitchell AA, Werler MM. Maternal genitourinary infections and the risk of gastroschisis. Am J Epidemiol. 2014;180(5):518-525.
16. James AH, Brancazio LR, Price T. Aspirin and reproductive outcomes. Obstet Gynecol Surv. 2008;63(1):49-57.
17. Centers for Disease Control and Prevention. Organic solvents. https://www.cdc.gov/niosh/topics/organsolv/. Updated 2017. Accessed 6/10, 2018.
18. Bruckner JV, Anand SS, Warren DA. Toxic effects of solvents and vapors. In: Klaassen CD, ed. Casarett and doull's toxicology: The basic science of poisons 7th edn. New York: McGraw-Hill; 2008:981-1051.

19. Spinder N, Prins JR, Bergman JEH, et al. Congenital anomalies in the offspring of occupationally exposed mothers: A systematic review and meta-analysis of studies using expert assessment for occupational exposures. Hum Reprod. 2019:5;903-919.

20. Torfs CP, Katz EA, Bateson TF, Lam PK, Curry CJ. Maternal medications and environmental exposures as risk factors for gastroschisis. Teratology. 1996;54(2):84-92.

21. Reefhuis J, Gilboa SM, Anderka M, et al. The national birth defects prevention study: A review of the methods. Birth Defects Res A Clin Mol Teratol. 2015;103(8):656-669.

22. Rasmussen SA, Olney RS, Holmes LB, et al. Guidelines for case classification for the national birth defects prevention study. Birth Defects Res A Clin Mol Teratol. 2003;67(3):193-201.

23. U.S. Bureau of Labor Statistics. 2010 SOC user guide. https://www.bls.gov/soc/soc_2010_user_guide.pdf. Updated 2010. Accessed 3/21, 2019.

24. Rocheleau CM, Lawson CC, Waters MA, et al. Inter-rater reliability of assessed prenatal maternal occupational exposures to solvents, polycyclic aromatic hydrocarbons, and heavy metals. J Occup Environ Hyg. 2011;8(12):718-728.

25. Samanic CM, De Roos AJ, Stewart PA, Rajaraman P, Waters MA, Inskip PD. Occupational exposure to pesticides and risk of adult brain tumors. Am J Epidemiol. 2008;167(8):976-985.

26. Rocheleau CM, Bertke SJ, Lawson CC, et al. Factors associated with employment status before and during pregnancy: Implications for studies of pregnancy outcomes. Am J Ind Med. 2017;60(4):329-341.

27. Benke G, Sim M, Forbes A, Salzberg M. Retrospective assessment of occupational exposure to chemicals in community-based studies: Validity and repeatability of industrial hygiene panel ratings. Int J Epidemiol. 1997;26(3):635-642.

28. Cogswell ME, Bitsko RH, Anderka M, et al. Control selection and participation in an ongoing, population-based, case-control study of birth defects: The national birth defects prevention study. Am J Epidemiol. 2009;170(8):975-985.

29. Johnson CY, Rocheleau CM, Grajewski B, Howards PP. Structure and control of healthy worker effects in studies of pregnancy outcomes. Am J Epidemiol. 2018;188(3):562-569.

30. Herdt-Losavio ML, Lin S, Chapman BR, et al. Maternal occupation and the risk of birth defects: An overview from the national birth defects prevention study. Occup Environ Med. 2010;67(1):58-66.

SUPPLEMENTARY MATERIAL

Supplementary Table 1 | Intensity scores for individual solvents

	Exposure intensity in ppm (Stoddard solvents in mg/m³)			
Solvent classes	Very low	Low	Medium	High
Aromatic solvents				
Benzene	<0.01	0.01 ≤ 0.1	0.1 ≤ 1	≥ 1
Toluene	<0.35	0.35 ≤ 3.5	3.5 ≤ 30	≥ 30
Xylene	<1	1 ≤ 5	5 ≤ 10	≥ 10
Chlorinated solvents				
Carbon tetrachloride	<0.1	0.1 ≤ 1	1 ≤ 10	≥ 10
Chloroform	<0.6	0.6 ≤ 6	6 ≤ 30	≥ 30
Methylene chloride	<0.35	0.35 ≤ 3.5	3.5 ≤ 30	≥ 30
Perchloroethylene	<0.2	0.2 ≤ 2	2 ≤ 15	≥ 15
1,1,1-trichloroethane	<6	6 ≤ 60	60 ≤ 300	≥ 300
Trichloroethylene	<0.35	0.35 ≤ 3.5	3.5 ≤ 30	≥ 30
Stoddard solvents	<6	6 ≤ 60	60 ≤ 300	≥ 300

Supplementary Table 2 | Correlation between solvent classes assessed in controls only

Solvent classes	Aromatic solvents	Chlorinated solvents	Stoddard solvents
Aromatic solvents	1	0.38	0.36
Chlorinated solvents	0.38	1	0.43
Stoddard solvents	0.36	0.43	1

Correlation coefficient assessed with Spearman's rho.

Supplementary Table 3 | Baseline characteristics of exposed and non-exposed control infants, National Birth Defects Prevention Study, USA, 1997-2011

	Exposed controls (n= 579)		Non-exposed controls (n = 7238)	
	N	(%)	N	(%)
Maternal age at delivery (years) Φ				
<20	24	(4.1%)	467	(6.5%)
20-24	152	(26.3%)	1594	(22.0%)
25-29	184	(31.8%)	2053	(28.4%)
30-34	148	(25.6%)	2015	(27.8%)
≥35	71	(12.3%)	1105	(15.3%)
Maternal education Φ				
≤12 years	236	(40.8%)	2265	(31.4%)
>12 years	342	(59.2%)	4955	(68.6%)
Maternal race-ethnicity				
Non-Hispanic white	359	(62.0%)	4641	(64.2%)
Non-Hispanic black	68	(11.7%)	831	(11.5%)
Hispanic	106	(18.3%)	1325	(18.3%)
Other	46	(7.9%)	436	(6.0%)
Pre-pregnancy BMI (kg/m^2)				
Underweight (<18.5)	22	(3.9%)	329	(4.6%)
Normal weight (18.5-25)	331	(59.1%)	3792	(53.5%)
Overweight (25-30)	119	(21.3%)	1652	(23.3%)
Obese (>30)	88	(15.7%)	1314	(18.5%)
Parity Φ				
0	237	(40.9%)	3277	(45.3%)
≥1	342	(59.1%)	3942	(54.7%)
Maternal cigarette smoking during periconceptional period[a] Φ				
Yes	229	(39.6%)	2268	(31.4%)
No	350	(60.4%)	4963	(68.6%)
Maternal alcohol use during periconceptional period Φ				
Yes	229	(39.6%)	2268	(31.4%)
No	350	(60.4%)	4948	(68.6%)
Maternal illicit drug use during periconceptional period[b]				
Yes	31	(5.4%)	298	(4.1%)
No	548	(94.6%)	6933	(95.9%)

Totals do not add up due to missing data. BMI = body mass index. [a] = self-reported cigarette smoking and second-hand cigarette smoke exposure at work and at home, [b] = included marijuana, hash, cocaine, crack, hallucinogens, heroin, and mushrooms, Φ = significant difference between exposed and non-exposed controls (p-value <0.05) using Chi Square tests.

Supplementary Table 4 | Estimated intensity of maternal occupational solvents exposure during the periconceptional period[a] and risk of gastroschisis in offspring, National Birth Defects Prevention Study, USA, 1997-2011

	Gastroschisis cases (n= 879)		Total Controls (n = 7817)		Unadjusted		Adjusted[b]	
Solvent classes	N	(%)	N	(%)	OR	95% CI	OR	95% CI
Any solvent								
No exposure	823	(93.6%)	7359	(94.1%)	Ref		Ref	
Very low	28	(3.2%)	225	(2.9%)	1.11	0.75 – 1.66	1.22	0.80 – 1.86
Low	19	(2.2%)	118	(1.5%)	1.44	0.88 – 2.35	1.17	0.70 – 1.97
Medium	7	(0.8%)	103	(1.3%)	0.61	0.28 – 1.31	0.69	0.31 – 1.53
High	<3				NC		NC	
Aromatic solvents								
No exposure[c]	860	(97.8%)	7651	(97.9%)	Ref		Ref	
Very low	9	(1.0%)	47	(0.6%)	1.70	0.83 – 3.49	2.25	1.03 – 4.90
Low	6	(0.7%)	62	(0.8%)	0.86	0.37 – 2.00	0.75	0.31 – 1.80
Medium	<3				NC		NC	
High	<3				NC		NC	
Chlorinated solvents								
No exposure[c]	836	(95.1%)	7513	(96.1%)	Ref		Ref	
Very low	25	(2.8%)	187	(2.4%)	1.20	0.79 – 1.84	1.42	0.91 – 2.23
Low	10	(1.1%)	60	(0.8%)	1.50	0.76 – 2.94	1.07	0.53 – 2.17
Medium	7	(0.8%)	54	(0.7%)	1.17	0.53 – 2.57	1.12	0.49 – 2.56
High	<3				NC		NC	
Stoddard solvents								
No exposure[c]	859	(97.8%)	7658	(98.0%)	Ref		Ref	
Very low	4	(0.5%)	47	(0.6%)	0.76	0.27 – 2.11	0.54	0.19 – 1.55
Low	13	(1.5%)	76	(1.0%)	1.53	0.84 – 2.76	1.27	0.68 – 2.38
Medium	<3				NC		NC	
High	<3				NC		NC	

Totals do not add up due to missing data. [a] = one month before conception through three months after conception, [b] = adjusted for maternal age at delivery as a continuous variable (no missing values), [c] = no exposure for outcome under analysis.

Supplementary Table 5 | Estimated frequency of maternal occupational solvents exposure during the periconceptional period[a] and risk of gastroschisis in offspring, National Birth Defects Prevention Study, USA, 1997-2011

	Gastroschisis cases (n= 879)		Total Controls (n = 7817)		Unadjusted		Adjusted[b]	
Solvent classes	N	(%)	N	(%)	OR	95% CI	OR	95% CI
Any solvent								
No exposure	815	(92.7%)	7238	(92.6%)	Ref		Ref	
0 -10 hours per week	52	(5.9%)	471	(6.0%)	0.98	0.73 – 1.32	1.07	0.78 – 1.45
>11 hours per week	23	(1.4%)	108	(1.4%)	0.99	0.54 – 1.80	0.77	0.41 – 1.44
Aromatic solvents								
No exposure[c]	860	(97.8%)	7651	(97.9%)	Ref		Ref	
0 -10 hours per week	16	(1.8%)	133	(1.7%)	1.07	0.63 – 1.81	1.23	0.71 – 2.15
>11 hours per week	3	(0.3%)	30	(0.4%)	0.89	0.27 – 2.92	0.85	0.24 – 2.95
Chlorinated solvents								
No exposure[c]	821	(93.6%)	7311	(93.6%)				
0 -10 hours per week	48	(5.5%)	428	(5.5%)	1.00	0.73 – 1.36	1.05	0.76 – 1.45
>11 hours per week	8	(0.9%)	73	(0.9%)	0.98	0.47 – 2.03	0.70	0.33 – 1.50
Stoddard solvents								
No exposure[c]	859	(97.8%)	7658	(98.0%)	Ref		Ref	
0 -10 hours per week	12	(1.4%)	115	(1.5%)	0.93	0.51 – 1.69	0.75	0.40 – 1.40
>11 hours per week	7	(0.8%)	43	(0.6%)	1.45	0.65 – 3.24	1.06	0.46 – 2.46

Totals do not add up due to missing data. [a] = one month before conception through three months after conception, [b] = adjusted for maternal age at delivery as a continuous variable (no missing values), [c] = no exposure for outcome under analysis.

CHAPTER 7

General discussion

Embryonic development is a complex process involving genetic, epigenetic, and environmental factors. Disturbances in embryonic development can lead to congenital anomalies, yet the aetiology of congenital anomalies is still not fully understood. In the Netherlands, an increasing number of women are working during their reproductive years and their pregnancies, which means that exposures in the workplace can have potential teratogenic effects. This thesis aimed to examine the associations between maternal occupational exposures early in pregnancy and the risks of congenital anomalies in offspring. The present chapter discusses the main findings and methodological challenges of this thesis and gives suggestions for future research and perspectives on periconceptional occupational health.

THE EFFECT OF MATERNAL OCCUPATIONAL EXPOSURES ON CONGENITAL ANOMALY DEVELOPMENT

This thesis focused on maternal occupational exposure to organic and mineral dusts, gases and fumes, solvents, pesticides, metals, and endocrine disrupting chemicals (EDCs). Approximately 35% of the women studied in this thesis were exposed to one or more of these occupational exposures. Table 1 summarizes the results of the research described in this thesis. Exposure to organic dust increased the risk of orofacial clefts in the offspring (Chapter 3), and occupational exposure to mineral and organic dusts and metals increased the risk of specific congenital heart defects (CHDs) (Chapter 5). Occupational exposure to solvents possibly increased the risk of neural tube defects, CHDs (Chapter 2), and urinary defects (Chapter 4), whereas exposure to pesticides was associated with a slightly higher prevalence of orofacial clefts in offspring (Chapter 3). Exposure to specific EDCs, such as phthalates, benzophenones, parabens, or siloxanes, increased the number of infants born with urinary anomalies (Chapter 4).

During their work, women were most likely exposed through inhalation and/or dermal contact to agents that could end up in the circulatory system after uptake. A recent study showed that inhaled fine particles are able to cross the placental barrier and are found at the foetal side of the placenta [1]. Once agents cross the placental barrier, exposure can induce oxidative stress, which may induce teratogenesis via misregulation of critical pathways involving foetal development [2].

Table 1 | Summary of the associations between maternal occupational exposures and congenital anomalies examined in this thesis

		Congenital anomalies					
Occupational exposures		Neural tube defects	Congenital heart defects	Orofacial clefts	Hypospadias	Urinary anomalies	Gastroschisis
Organic dust			=	+			
Mineral dust			+	=			
Gases and fumes				=			
Metals	Meta-analysis	NC	+	+	NC		
			+	=			
Solvents	Meta-analysis	+	+	NC	NC		
			=	=	=	+	=
Pesticides	Meta-analysis	=	=	NC	=		
			=	+	-	-	
PAHs					+	=	
Phthalates / Benzophenones / Parabens / Siloxanes					+	+	

-/=/+ represent the estimate (adjusted odds ratio (OR)) or pooled estimate for the meta-analyses
- OR <0.8; = OR ≥0.8 and <1.2; + OR ≥1.2. NC = pooled OR not calculated due to heterogeneity.
Red values represent significant values (p<0.05).
Associations represented by empty cells were not examined in this thesis. Analyses for subgroups of congenital anomalies and subgroups of occupational exposure are not displayed.

Exposure to organic and mineral dusts

In this thesis, exposures to organic and mineral dusts were relatively common, with about 30% and 10% of female employees being exposed, respectively. Organic dust is defined as dusts from plants (vegetables), animals, wood, or microbes, while mineral dust originates from minerals in soil. Mothers working as personal care workers, nursing professionals, cleaners, and agricultural workers were those most likely exposed to organic dust. Cleaners and agricultural workers were also considered to be simultaneously exposed to mineral dust. In Chapter 3, we showed that maternal occupational exposure to organic dust increased the risk of orofacial clefts (specifically cleft lip with or without palate). Mineral dust appeared to be associated with a higher risk for specific CHDs, such as coarctation of the aorta and pulmonary (valve) stenosis (Chapter 5). No other studies were identified that studied the effect of organic and mineral dusts on reproductive health and, especially, foetal development.

Exposure to metals

In this thesis, fewer than 1% of working mothers were exposed to metals. Occupational metal exposure occurs through exposure to metal dusts and fumes produced during metalworking processes (grinding, welding, cutting, lathe work, etc.). The women included

in this analysis worked as electronic-equipment or mechanical-machinery assemblers. In Chapter 5, an association between maternal occupational exposure to metals and CHDs was observed. In Chapter 2, three previous studies regarding maternal occupational exposure to metals and CHDs were pooled in the meta-analysis, and no association was found. However, we were not able to explore subgroups of CHDs in this meta-analysis even though it is important to assess subgroups because specific CHDs are anatomically, clinically, epidemiologically, and developmentally heterogeneous [3]. In Chapter 5, we were able to perform subgroup analysis for specific heart defects and found that maternal occupational exposure to metals is particularly associated with isolated septal defects.

Exposure to solvents

Approximately 7-20% of the women studied in this thesis were exposed to solvents during their work. Solvents are present in paints, adhesives, glues, and degreasing/cleaning agents. Women occupationally exposed to solvents mainly worked in healthcare, beauty or hairdressing salons, or as cleaners.

The meta-analysis conducted in Chapter 2 showed that maternal occupational exposure to solvents increased the risk of neural tube defects in offspring. All studies using expert-based occupational exposure assessment regarding maternal occupational exposure to solvents and neural tube defects were included in this meta-analysis. Our observed association is supported by studies that found associations between neural tube defects and maternal jobs that were likely exposed to solvents, such as jobs in healthcare (nurses), cleaning, chemical sciences, and agriculture [4-6].

In Chapter 5, no association between maternal occupational exposure to solvents and CHDs was observed. In the meta-analysis, an association between solvent exposure and CHDs was observed (Chapter 2). It is important to assess CHDs in subgroups, e.g. using the Botto classification [3], because of their anatomical, clinical, epidemiological, and developmental differences. In Chapter 5, we were able to perform such a subgroup analyses and found no association between maternal occupational exposure to solvents and any of the CHD subgroups. We were not able to perform subgroup analyses in the meta-analysis.

In Chapter 4, an association was found between maternal occupational exposure to solvents that have an endocrine disrupting effect and urinary anomalies, particularly for anomalies of the urinary collecting system and when more than one urinary anomaly was present. One earlier study had also found an association between solvent exposure and

urinary anomalies [7], but the results of two other studies were not in line with our finding [8,9]. However, the number of urinary cases included in these previous studies was low compared to the study described in this thesis (n=12 and n=76 versus n=537 here), and no subgroup analyses were performed.

Exposure to pesticides

In this thesis, only 2-3% of women participating in the labour force were exposed to pesticides. Women were most likely to be exposed to pesticides when they were working in the agricultural sector. In Chapter 3, an increased risk of developing an orofacial cleft was found among offspring of women with occupational exposure to pesticides. In our meta-analysis, we could not calculate a pooled estimate for pesticide exposure and orofacial clefts because the two studies included were too heterogeneous to calculate a pooled estimate (Chapter 2). A previous meta-analysis that examined this association and included more studies had suggested that exposure to pesticides can lead to a modest increase of orofacial clefts in the offspring [10]. However, most of the studies they included relied on self-reported exposure, which might have biased the association upward.

METHODOLOGICAL CHALLENGES

Occupational exposure assessment methods

In this thesis two occupational exposure assessment methods were used: group-based job-exposure matrices (JEMs) and individual-based expert assessments by occupational hygienists. Both methods are retrospective and based primarily on self-reported job characteristics early in pregnancy or during the periconceptional period. An advantage of both assessment methods is that they limit recall bias and differential misclassification of exposure [11].

A limitation of the JEMs used in this thesis is that non-differential misclassification is introduced in two different ways. First, the JEM does not account for the time period in which the job was performed, and previous studies have shown that occupational exposure can vary over time [12,13]. Second, circumstances at the workplace are often unpredictable and can vary within a job and between companies [11]. In individual-based assessment, occupational hygienists can take differences over time, within jobs, and between companies into consideration. They can rely on information available for a wide variety of occupational characteristics to perform a more detailed exposure assessment

at individual level, examining factors such as job title, employer name, what the company produces, primary tasks and duties, a description of chemicals and machines handled on the job, dates of employment, and hours and days worked per week. However, a challenge of assessing occupational exposure at the individual level is that the questionnaires for participants need to include more specific questions on tasks and circumstances for a wide variety of jobs, resulting in long complicated questionnaires for participants. Another limitation of this method is that it is time-consuming and requires expensive input from an occupational hygienist.

A limitation in Chapters 3-5 was that correction for the number of hours and weeks mothers worked early in pregnancy was not possible due to absence of this information. In Chapter 6, we were able to account for the dates of employment and hours and days worked per week during the periconceptional period.

Finally, measurement error is unavoidable for both assessment methods. Individual-based exposure assessment by occupational hygienists could result in classical error because the exposure assigned at an individual level will vary around a true value depending on the quality of the self-reported information. Reporting bias could also be an issue. In contrast, a JEM assigns exposure at job level rather than individual level, and this exposure assignment will therefore produce risk estimates with no bias or only minor bias, but this will come with a loss of precision, generally known as a Berkson type error [14].

A special time window of exposure: early in pregnancy

In this thesis, maternal occupational exposure was assessed during a special time window in a woman's life: early in pregnancy. While some women will be very careful during this time because they are pregnant after a long period of fertility treatments, other pregnancies are unplanned or unexpected, and these women will initially not even know they are pregnant. Therefore, it is possible that some women avoided certain exposures because they wanted to become pregnant, or knew they were pregnant, while performing a job that includes exposure. Another possibility could be that women avoided or were advised by their employer not to handle certain solvents or other chemicals during work, or wore protective equipment, because they wanted to become pregnant or knew they were pregnant. An exposure assessment based on precise information about how women behaved early in pregnancy, when they informed their employer, and if, when and by whom preventive measures were taken would result in less misclassification.

A Dutch report published in 2007 showed that protective measures were taken by the employer for only 40-50% of the pregnant women working with harmful chemicals [15]. This report did not show at which gestational age measures were taken, which is important since organogenesis is already complete at the end of the first trimester. Unfortunately, there is no more recent literature regarding occupational behaviour of pregnant women or women who want to become pregnant in the Netherlands. Although the report was published more than a decade ago, protection of pregnant women is probably still not fully incorporated into workplace practices, as pregnancy discrimination is prevalent, with more than 40% of working women reporting negative experiences with their pregnancy in relation to work [16]. Additionally, for female freelancers or self-employed contractors, it might be difficult to avoid exposure. More up-to-date information is needed to give a more precise estimate of the actual exposures of women in the periconceptional period. This is could be done by asking women when they found out they were pregnant, if and when they changed their occupational behaviour, when they informed their employer, and when were preventive measures taken.

Surveillance and registration of infants with congenital anomalies

Surveillance and registration of congenital anomalies is complex because 2-3% of pregnancies worldwide are affected by a congenital anomaly and subgroups of congenital anomalies are very different in aetiology and relatively uncommon. In this thesis, high quality data from Eurocat Northern Netherlands (Eurocat NNL) and the National Birth Defects Prevention Study (NBDPS) were used. Eurocat NNL has detailed medical information available for each case, and all cases were coded by trained registry staff according to international coding guidelines [17]. The NBDPS abstracted clinical information from medical records, which was then reviewed by clinical geneticists using a systematic study-wide classification protocol [18]. The registration of congenital anomalies is important because, through detailed epidemiological surveillance of congenital anomalies over a long time period, reliable information can be generated about possible increases in numbers of congenital anomalies in order to detect a new epidemic as soon as possible. Active surveillance can also reassure or support clinicians if they detect a possible cluster of congenital anomalies. One strength of surveillance using high quality data is that homogenous and detailed groups of congenital anomalies could be examined to study risk factors, such as the occupational exposure examined in this thesis.

Despite the detailed information available for each infant, several analyses in this thesis using data from Eurocat NNL included a low number of infants affected by very specific anomalies. The catchment area of Eurocat NNL is limited to the three Northern provinces. To increase the power of these studies, it would be helpful to extend the coverage and methods used by Eurocat NNL to the whole of the Netherlands, or even to the whole of Europe. Unfortunately, it was not possible to use the international Eurocat Network in this thesis because not all European registries have information on maternal occupation during the periconceptional period.

Another challenge for both Eurocat and the NBDPS was collecting data for all infants born with a congenital anomaly. In Eurocat NNL, approximately three-fourths of parents gave permission for registration and, of those, approximately two-thirds filled in the questionnaire. In the NBDPS, two-thirds of the invited women participated. This could have introduced selection bias because it is known that people who do not participate in scientific studies have, on average, a lower socioeconomic status, with more potential for occupational exposure, and are more likely to live in urban environments as compared to participants [19,20]. To improve the surveillance of congenital anomalies in the Northern Netherlands, Eurocat NNL is allowed to register limited information on the anomaly if parents who have an infant born with a congenital anomaly do not respond to the invitation to participate. However, data on risk factors are not registered in these cases.

Selection of controls

In this thesis, four different control groups were used. The definition of the ideal control group is that controls should be free of the disease being studied and represent the population at risk of becoming cases [21]. In Chapter 6, the control group used is consistent with this definition. The NBDPS randomly selected live-born infants without major congenital anomaly (non-malformed controls) from vital records or birth hospital records from the same geographical region and time period as cases [18]. The control participants of the NBDPS are representative of their base population [22]. However, this method is time-consuming and expensive, as significantly more infants have to be identified and recruited, and more information needs to be collected. Additionally, recall bias could have been an issue, since parents who have an infant with a congenital anomaly may search their memories more thoroughly for exposures to possible risk factors than parents who have a healthy infant. Because Eurocat NNL does not collect data on non-malformed controls, three other control groups were used in Chapter 3-5.

In Chapter 3, two control groups were created: chromosomal malformed controls (infants with a chromosomal/monogenic anomaly born during the same time period in the same geographical region as cases) and non-chromosomal malformed controls (all infants with a non-chromosomal/non-monogenic congenital anomaly, but not affected by the anomaly under study, born during the same time period in the same geographical region as cases). From a historical perspective, Eurocat NNL has been using chromosomal controls in case–control studies examining risk factors for congenital anomalies. A genetic cause is identified for those anomalies, implying that other causes, for example from the environment, were unlikely. There is some evidence that occupational exposure to pesticides can have a mutagenic effect and that mineral dust can induce DNA methylation in humans [23,24]. Another study showed that women living near solvent and metal waste sites have an increased risk of chromosomal anomalies in offspring [25]. A recent study suggests that maternal occupational exposure to solvents among production workers increased the risk of chromosome 21 nondisjunction, resulting in trisomy 21 [26]. Based on those studies, it seems possible that environmental factors might increase the risk of chromosomal/monogenic anomalies. As a consequence, the use of chromosomal malformed controls could result in underestimation of risk estimates of maternal occupational exposure on development of congenital anomalies. Therefore, a second control group was created of infants with non-chromosomal anomalies. However, it is known that maternal occupational exposures could increase the risk of several congenital anomalies included in this control group. Therefore analysis with infants with non-chromosomal anomalies as controls could have introduced bias resulting in an underestimation of the effect.

Infants without congenital anomaly were selected from the general population Lifelines cohort for the studies described in Chapter 4 and 5. However, this method could have introduced selection bias, because individuals with a higher socioeconomic status are more likely to participate in a biobank such as Lifelines [19,20]. Nevertheless, a previous study showed that the Lifelines cohort is a representative sample of the population in the Northern Netherlands [27]. As discussed above, selection bias is also possible for cases/malformed controls because parents with a higher socioeconomic status could be overrepresented amongst those who filled in the Eurocat questionnaire. Another concern in using non-malformed controls from the general population Lifelines cohort is that information bias could have been introduced due to differences in questionnaires and the timing of questionnaires. Additionally, recall bias could have been an issue in the same way described above for the NBDPS healthy control group. For occupational exposure,

information bias and recall bias is unlikely, since mothers participating in both Eurocat and Lifelines were asked about their job early in pregnancy and the JEM assigns exposure based on job only. However, information bias and recall bias might have been an issue some for important covariates, such as folic acid use, smoking, alcohol use, and body mass index.

To gain more insight into the effect of the different control groups, a post-hoc analysis was performed for the orofacial cleft study described in Chapter 3. In this study, we examined the association between maternal occupational exposures and orofacial clefts compared to a chromosomal and a non-chromosomal malformed control group from Eurocat. This analysis was repeated with non-malformed controls from the Lifelines cohort. Effect estimates turned out to be of the same magnitude and in the same direction for all three control groups.

RECOMMENDATIONS

Based on the results and challenges described in the previous sections, directions for future research are given below. In addition, we provide recommendation for female workers who want to become pregnant, or are pregnant, and for employers.

Methodological recommendation for future research

Based on the methodological challenges described above, we can make recommendations for future case–control studies that want to examine risk factors for congenital anomalies. We recommend recruiting controls together with cases. Control infants should be infants without congenital anomalies from the same geographical area and time-period as cases. For Eurocat NNL, it is not feasible to collect data on non-malformed infants because Eurocat NNL has only been collecting data on risk factors since 1997 and retrospectively including non-malformed infants would have limitations as well (e.g. introducing recall bias). The recommendation for Eurocat NNL case–control studies is to select non-malformed control infants from a cohort of infants without congenital anomalies, such as Lifelines. Analyses should be performed with a second control group consisting of chromosomal malformed controls from Eurocat NNL to account for bias introduced using the non-malformed control group.

In future studies, occupational exposure assessment should be performed by occupational hygienists, and questionnaires should include questions on a wide variety of occupational variables. A JEM is a good and far less-costly alternative if only the job description is known.

Studies, including those of Eurocat NNL, must consider extending their questionnaire with additional questions about the number of hours and weeks mothers worked early in pregnancy because, in contrast to other European countries, the majority of women in the Netherlands hold part-time jobs [28,29].

Topics for future research

In the Netherlands, employers are required by law to protect pregnant employees and their unborn child from adverse occupational effects, but there is no recent information on the effectiveness of reproductive health policies. We therefore recommend studying how women behave early in pregnancy: Do they acknowledge occupational risks? If, when, and by whom are preventive measures taken at work? When do women inform their employer about the pregnancy? Do they feel they are working in a safe environment? This will allow for the detection of knowledge gaps and reveal better ways of protecting pregnant working women and those who want to become pregnant.

Future research should consider paternal occupational exposure as well, with several studies suggesting that paternal occupational exposure can increase the risk of congenital anomalies in the offspring [30-33]. Paternal exposure to chemicals could induce structural, genetic and/or epigenetic abnormalities in the sperm. However, there is currently no clear relation between the sperm abnormalities and offspring health [34].

In addition to paternal exposure, future research should work towards risk prediction models that incorporate several risk factors, as most congenital anomalies do not develop through exposure to a single risk factor. These models can then be used to identify high-risk groups in the population. In the United States, a risk prediction model for neural tube defects was not able to successfully identify high risk groups [35]. However, prediction models developed in China identified groups at high risk for CHDs [36]. Other prediction models have already been developed and implemented successfully to predict the risk of pregnancy complications in the Netherlands. One successful prediction model facilitated risk-based care, which reduced perinatal adverse outcomes in nulliparous women [37].

In addition to environmental exposures, genetic risk factors should also be included in risk models. This could be achieved by performing gene–environment interaction studies [38]. The NBDPS collected buccal cells to perform genetic analysis [18]. This study is extended through the Birth Defects Study To Evaluate Pregnancy exposureS (BD-STEPS), which requested permission to sample residual newborn screening blood spots for genetic

analysis [39]. The international EUROCAT network should also consider collecting genetic information to perform gene–environment interactions studies.

Periconceptional occupational health: advice for the female workforce and their employers

Working women and employers must be aware that the periconceptional period is a crucial period for giving birth to a healthy child. Maternal occupational exposure to organic dust and solvents early in pregnancy increases the risk of orofacial clefts, neural tube defects, urinary defects, and CHDs in offspring. As these exposures are common in the population, they can be significant contributors to the risk of developing those anomalies. Maternal exposure to mineral dust, pesticides, and metals increases the risk of orofacial clefts and CHDs. As exposure to these agents is currently uncommon among women in the Netherlands, the public health impact will be limited.

Women must be aware of the possible risk that occupational exposure can have on development of congenital anomalies in their offspring. They should be aware of their exposure to organic or mineral dusts, solvents, pesticides, or metals during their work, and of how adequate protective measures can be taken.

As Dutch law requires, employers are obligated to identify risks for pregnant employees and to inform employees about these risks. Employers must create a safe working environment and limit possible teratogenic exposures. Employees and employers must also ensure that they work in accordance with the protocols, which will reduce occupational exposure and therefore possibly reduce the risk of congenital anomalies in offspring. Together, it is essential that employees and employers consult an occupational hygienist/physician when needed.

CONCLUSION

This thesis has shown that maternal occupational exposure to organic dust and solvents early in pregnancy is relatively common and increases the risk of orofacial clefts, neural tube defects, urinary defects, and CHDs. Maternal exposures to mineral dust, pesticides, and metals are less prevalent, but increase the risk of orofacial clefts and CHDs. Employers should perform careful risk inventories and evaluations at their workplace, if necessary with input from an occupational hygienist. The female workforce should be informed about their occupational exposures and educated about recommended policies to

limit teratogenic exposure as much as possible in order to reduce the risk of congenital anomalies in offspring. Employees and employers should not hesitate to consult and discuss uncertainties with occupational hygienists and/or occupational physicians.

Future research should employ occupational exposure assessment methods that take into account the amount of data about occupational characteristics they foresee collecting, as group-based JEMs and individual-based expert assessments by occupational hygienists have different strengths and limitations and require different budgets. Control group selection should depend on the study population. However, researchers must be aware of the types of bias that could be introduced by using different types of control groups. Future research should investigate the current effectiveness of reproductive health policies and the occupational behaviour of pregnant women. Since many congenital anomalies are the result of the combined effects of genetics and maternal and paternal environmental factors, gene–environment interaction studies should be performed. The outcome of these studies could eventually lead to risk prediction models that will enable identification of groups in the population at high risk for congenital anomalies, thereby allowing for better protection and prevention and consequently fewer congenital malformations.

REFERENCES

1. Bové H, Bongaerts E, Slenders E, et al. Ambient black carbon particles reach the fetal side of human placenta. Nature communications. 2019;10(1):3866.
2. Hansen JM. Oxidative stress as a mechanism of teratogenesis. Birth defects research.Part C, Embryo today : reviews. 2006;78(4):293-307.
3. Botto LD, Lin AE, Riehle-Colarusso T, Malik S, Correa A, National Birth Defects Prevention Study. Seeking causes: Classifying and evaluating congenital heart defects in etiologic studies. Birth Defects Res A Clin Mol Teratol. 2007;79(10):714-727.
4. Blatter BM, Roeleveld N. Spina bifida and parental occupation in a Swedish register-based study. Scand J Work Environ Health. 1996;22(6):433-437.
5. Kim J, Langlois PH, Mitchell LE, Agopian AJ. Maternal occupation and the risk of neural tube defects in offspring. Arch Environ Occup Health. 2017:1-9.
6. Lin S, Herdt-Losavio ML, Chapman BR, et al. Maternal occupation and the risk of major birth defects: A follow-up analysis from the national birth defects prevention study. Int J Hyg Environ Health. 2013;216(3):317-323.
7. Garlantézec R, Monfort C, Rouget F, Cordier S. Maternal occupational exposure to solvents and congenital malformations: A prospective study in the general population. Occup Environ Med. 2009;66(7):456-463.
8. Cordier S, Bergeret A, Goujard J, et al. Congenital malformations and maternal occupational exposure to glycol ethers. Epidemiology. 1997;8(4):355-363.
9. Cordier S, Szabova E, Fevotte J, Bergeret A, Plackova S, Mandereau L. Congenital malformations and maternal exposure to glycol ethers in the Slovak republic 5]. Epidemiology. 2001;12(5):592-593.
10. Romitti PA, Sun L, Honein MA, Reefhuis J, Correa A, Rasmussen SA. Maternal periconceptional alcohol consumption and risk of orofacial clefts. Am J Epidemiol. 2007;166(7):775-785.
11. Teschke K, Olshan AF, Daniels JL, et al. Occupational exposure assessment in case-control studies: Opportunities for improvement. Occup Environ Med. 2002;59(9):575-93; discussion 594.
12. Burstyn I, Kromhout H. Trends in inhalation exposure to hydrocarbons among commercial painters in the Netherlands. Scand J Work Environ Health. 2002;28(6):429-438.
13. Peters S, Vermeulen R, Portengen L, et al. Modelling of occupational respirable crystalline silica exposure for quantitative exposure assessment in community-based case-control studies. J Environ Monit. 2011;13(11):3262-3268.
14. Heid IM, Kuchenhoff H, Miles J, Kreienbrock L, Wichmann HE. Two dimensions of measurement error: Classical and Berkson error in residential radon exposure assessment. J Expo Anal Environ Epidemiol. 2004;14(5):365-377.
15. Hooftman W, van den Bossche S. Zwangerschap en werk: Voorlichting, maatregelen en verzuim: Deelresultaten nationale enquête arbeidsomstandigheden 2003-2006. Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek TNO. 2007.

16. College voor de Rechten van de Mens. Zwangerschapsdiscriminatie is een probleem dat helaas nog niet minder wordt. . Feb 19, 2020. Available from: https://www.mensenrechten.nl/nl/toegelicht/zwangerschapsdiscriminatie-een-probleem-dat-helaas-nog-niet-minder-wordt. Accessed March 28, 2020.

17. Greenlees R, Neville A, Addor MC, et al. Paper 6: EUROCAT member registries: Organization and activities. Birth Defects Res A Clin Mol Teratol. 2011;91 Suppl 1:S51-S100.

18. Reefhuis J, Gilboa SM, Anderka M, et al. The national birth defects prevention study: A review of the methods. Birth Defects Res A Clin Mol Teratol. 2015;103(8):656-669.

19. Banks E, Herbert N, Mather T, Rogers K, Jorm L. Characteristics of Australian cohort study participants who do and do not take up an additional invitation to join a long-term biobank: The 45 and up study. BMC research notes. 2012;5:655.

20. Langhammer A, Krokstad S, Romundstad P, Heggland J, Holmen J. The HUNT study: Participation is associated with survival and depends on socioeconomic status, diseases and symptoms. BMC medical research methodology. 2012;12:143.

21. Schulz KF, Grimes DA. Case-control studies: Research in reverse. Lancet (London, England). 2002;359(9304):431-434.

22. Cogswell ME, Bitsko RH, Anderka M, et al. Control selection and participation in an ongoing, population-based, case-control study of birth defects: The national birth defects prevention study. Am J Epidemiol. 2009;170(8):975-985.

23. Bolognesi C. Genotoxicity of pesticides: A review of human biomonitoring studies. Mutat Res. 2003;543(3):251-272.

24. van der Plaat, D. A., Vonk JM, Terzikhan N, et al. Occupational exposure to gases/fumes and mineral dust affect DNA methylation levels of genes regulating expression. Hum Mol Genet. 2019. doi: z067 [pii].

25. Brender JD, Zhan FB, Langlois PH, Suarez L, Scheuerle A. Residential proximity to waste sites and industrial facilities and chromosomal anomalies in offspring. Int J Hyg Environ Health. 2008;211(1-2):50-58.

26. Keen C, Hunter JE, Allen EG, Rocheleau C, Waters M, Sherman SL. The association between maternal occupation and down syndrome: A report from the national down syndrome project. Int J Hyg Environ Health. 2020;223(1):207-213.

27. Klijs B, Scholtens S, Mandemakers JJ, Snieder H, Stolk RP, Smidt N. Representativeness of the LifeLines cohort study. PLoS One. 2015;10(9):e0137203.

28. Sociaal en Cultureel Planbureau. Werken aan de start: Jonge vrouwen en mannen op de arbeidsmarkt. 2018:83-84.

29. Thor (CBS) Jv, Portegijs (SCP) W, Hermans (CBS) B. Gaan vrouwen steeds meer werken? Emancipatiemonitor: 2018. 2018. https://digitaal.scp.nl/emancipatiemonitor2018/gaan-vrouwen-steeds-meer-werken.

30. Kalfa N, Philibert P, Broussous S, et al. Isolated hypospadias and exposure to endocrine disrupting chemicals during pregnancy: A multi-institutional controlled study in a high prevalence area. Horm Res Paediatr. 2014;82:107.

31. Suhl J, Romitti PA, Rocheleau C, et al. Parental occupational pesticide exposure and nonsyndromic orofacial clefts. J Occup Environ Hyg. 2018;15(9):641-653.

32. Pettigrew SM, Bell EM, Van Zutphen AR, et al. Paternal and joint parental occupational pesticide exposure and spina bifida in the national birth defects prevention study, 1997 to 2002. Birth Defects Res A Clin Mol Teratol. 2016;106(11):963-971.

33. Wang C, Zhan Y, Wang F, et al. Parental occupational exposures to endocrine disruptors and the risk of simple isolated congenital heart defects. Pediatr Cardiol 2015;36(5):1024-37

34. Delbès G, Hales BF, Robaire B. Toxicants and human sperm chromatin integrity. Mol Hum Reprod. 2010;16(1):14-22.

35. Agopian AJ, Lupo PJ, Tinker SC, Canfield MA, Mitchell LE, and the National Birth Defects, Prevention Study. Working towards a risk prediction model for neural tube defects. Birth Defects Research Part A: Clinical and Molecular Teratology. 2012;94(3):141-146.

36. Luo Y, Li Z, Guo H, et al. Predicting congenital heart defects: A comparison of three data mining methods. PLoS One. 2017;12(5):e0177811.

37. van Montfort P, Scheepers HCJ, Dirksen CD, et al. Impact on perinatal health and cost-effectiveness of risk-based care in obstetrics: A before after study. Am J Obstet Gynecol. 2020.

38. Tai CG, Graff RE, Liu J, et al. Detecting gene-environment interactions in human birth defects: Study designs and statistical methods. Birth Defects Res A Clin Mol Teratol. 2015;103(8):692-702.

39. Tinker SC, Carmichael SL, Anderka M, et al. Next steps for birth defects research and prevention: The birth defects study to evaluate pregnancy exposures (BD-STEPS). Birth Defects Res A Clin Mol Teratol. 2015;103(8):733-740.

7

APPENDICES

SUMMARY

One in 33 infants worldwide is born with a congenital anomaly. Embryonic development is a complex process involving genetic, epigenetic, and environmental factors, and disturbances in embryonic development can lead to congenital anomalies. However, the aetiology of many congenital anomalies is not yet fully understood. In the Netherlands, an increasing number of women are working during their reproductive years and their pregnancies, which increases the chances of potential teratogenic effects due to exposures in the workplace. It is thus important to identify teratogenic exposures in order to protect women who want to become pregnant or are pregnant. This is crucial because having an infant with a congenital anomaly has a large impact, not only on the infant's health, but also on their families and society at large. Therefore, this thesis aimed to examine the association between maternal occupational exposures early in pregnancy and the risk of congenital anomalies in the offspring.

In **Chapter 2**, a systematic review and meta-analysis was performed to examine the association of maternal occupational exposure to solvents, pesticides, and metals with congenital anomalies in offspring. Four subgroups of congenital anomalies were examined: neural tube defects, congenital heart defects, orofacial clefts, and hypospadias. The results showed an association between maternal occupational exposure to solvents and neural tube defects, congenital heart defects, and orofacial clefts in offspring. No association was observed between maternal occupational exposure to pesticides or metals and congenital anomalies.

In **Chapter 3**, a case–control study was performed to assess maternal occupational exposure to solvents, pesticides, metals, dusts, and gases and fumes early in pregnancy in relation to orofacial clefts in offspring. Cases with an orofacial cleft (124 infants with cleft palate and 263 infants with cleft lip with or without cleft palate) were selected from the Eurocat Northern Netherlands (Eurocat NNL) population-based registry for congenital anomalies. Two control groups were selected from Eurocat NNL: (1) infants born with chromosomal/monogenic defects (n=1,135), and (2) infants born with non-chromosomal/non-monogenic congenital anomalies (n=4,356). In total, 44% of case mothers, 41.0% chromosomal and 37.7% of non-chromosomal control mothers were exposed to one of the agents considered in this study. The results indicated that maternal occupational exposure to pesticides and organic dust early in pregnancy are risk factors for orofacial clefts in offspring.

The development of the urogenital tract takes place under the influence of hormones. Exposure to endocrine disrupting chemicals (EDCs) can result in abnormal development of the urogenital tract. In **Chapter 4,** the association between maternal occupational exposure to EDCs early in pregnancy and urogenital anomalies in offspring was assessed in a case–control study. Infants born with urinary anomalies or hypospadias were selected from the Eurocat NNL registry. Urinary anomalies were classified into four groups: (I) malformations of the renal parenchyma, (II) anomalies of the urinary collecting system, (III) abnormal embryonic migration of kidneys and other urinary tract anomalies, and (IV) combinations of urinary anomalies. Controls without congenital anomaly were selected from the Lifelines cohort study. This study included 537 infants with urinary anomalies, 371 infants with hypospadias, and 5,602 controls, with 23% of case mothers and 20% of control mothers exposed to any EDC. The results of this study showed an association between maternal occupational exposure to some specific EDCs (organic solvents / alkylphenolic compounds and phthalates / benzophenones / parabens / siloxanes) and urinary anomalies, specifically anomalies of the urinary collecting system or when more than one urinary anomaly was present among cases.

In **Chapter 5**, the association between maternal occupational exposure to organic and mineral dust, solvents, pesticides, and metals was assessed in relation to congenital heart defects in offspring. Cases with congenital heart defects were selected from Eurocat NNL and classified into seven main subgroups to account for the diversity of cardiac phenotypes and underlying developmental mechanisms: conotruncal heart defects, atrioventricular septal defects, anomalous pulmonary venous return, left and right ventricular outflow tract obstructions, septal defects, and complex heart defects. Controls without a congenital anomaly were selected from the Lifelines cohort study. For the 1,174 cases with congenital heart defects and 5,602 controls, overall exposure to one or more maternal occupational exposures was 37.6% and 35.6%, respectively. The results of this study showed that maternal occupational exposures to organic dust, mineral dust, and metal dust and fumes early in pregnancy could increase the risk of left and right ventricular outflow tract obstructions and septal defects.

In **Chapter 6**, a case–control study was conducted that assessed the association between maternal occupational exposure to solvents and gastroschisis in the offspring. This study was performed in the United States using data from the National Birth Defects Prevention Study. This is a large population-based case–control study of major congenital anomalies that was conducted in ten states from 1997 to 2011. In total, 879 cases with gastroschisis

and 7,817 controls without congenital anomalies were included. The overall prevalence of occupational exposure to solvents was 7.3% in case mothers and 7.4% in control mothers during the periconceptional period (1 month before conception through 3 months after conception). No association was found between maternal occupational exposure to solvents and gastroschisis in the offspring, nor was an exposure–response relationship observed.

In **Chapter 7**, the main findings and methodological challenges in this thesis are discussed, followed by suggestions for future research and perspectives on periconceptional occupational health. Maternal occupational exposure to organic dust and solvents early in pregnancy is relatively common and increases the risk of orofacial clefts, neural tube defects, urinary defects, and congenital heart defects. Maternal exposures to mineral dust, pesticides, and metals are less prevalent, but increase the risk of orofacial clefts and congenital heart defects. Employers should perform careful risk inventories and evaluations at their workplace, if necessary with input from an occupational hygienist. The female workforce should be informed about their occupational exposures and educated about the recommended policies to limit teratogenic exposure as much as possible in order to reduce the risk of congenital anomalies in offspring. Employees and employers should not hesitate to consult and discuss uncertainties with occupational hygienists and/or occupational physicians.

Future research should employ occupational exposure assessment methods that account for the number and kind of occupational characteristics they foresee collecting, as group-based job exposure matrices and individual-based expert assessments by occupational hygienists have different strengths and limitations and require different budgets. Control group selection should be done based on the study population. However, researchers must be aware of the types of bias that can be introduced by using different types of control groups. Future research should also take into account the effectiveness of reproductive health policies and the occupational behaviour of pregnant women. Since many congenital anomalies are the result of the combined effects of genetics and maternal and paternal environmental factors, gene–environment interactions studies should be performed. The outcome of such studies should eventually lead to risk prediction models that will enable identification of groups in the population at high risk for congenital anomalies and lead to protection and prevention and consequently to fewer congenital malformations.

SAMENVATTING

Wereldwijd wordt één op de 33 kinderen geboren met een aangeboren aandoening. Foetale ontwikkeling is een complex proces dat beïnvloed wordt door genetische, epigenetische en omgevingsfactoren. Verstoring van de normale foetale ontwikkeling kan leiden tot aangeboren aandoeningen. Blootstelling aan schadelijke stoffen tijdens het werk gedurende de zwangerschap kan invloed hebben op de foetale ontwikkeling. Tegenwoordig werkt meer dan 80% van de Nederlandse vrouwen tijden hun reproductieve levensfase, 40 jaar geleden was dit nog maar 50% van de vrouwen. Hierdoor neemt de kans toe dat vrouwen tijdens hun werk worden blootgesteld aan mogelijk schadelijke effecten die de zich ontwikkelende foetus kunnen beïnvloeden. Het is daarom belangrijk om deze beroepsmatige reproductieve effecten te identificeren, zodat vrouwen die zwanger willen worden of zwanger zijn, blootstelling aan deze factoren kunnen voorkomen. Het krijgen van een kind met een aangeboren aandoening heeft veel impact, niet alleen op de gezondheid van het kind, maar ook op hun familie en de maatschappij. In dit proefschrift wordt het verband tussen maternale beroepsmatige blootstelling vroeg in de zwangerschap en het risico op het krijgen van een kind met een aangeboren aandoening onderzocht.

In **Hoofdstuk 2** is maternale beroepsmatige blootstelling aan oplosmiddelen, gewasbeschermingsmiddelen en metalen in relatie tot vier specifieke aangeboren aandoeningen onderzocht door middel van systematisch literatuuronderzoek en meta-analyse. De volgende vier aangeboren aandoeningen zijn hierin meegenomen: neuraal buis defecten (open ruggetje), hartafwijkingen, schisis (spleet in de bovenlip, kaak en/of het gehemelte) en hypospadie (plasbuis mondt uit aan de onderzijde van de penis). Dit review liet zien dat maternale beroepsmatige blootstelling aan oplosmiddelen een verhoogd risico geeft op neuraal buis defecten, hartafwijkingen en schisis bij het kind. Er werd geen verband gevonden tussen blootstelling aan gewasbeschermingsmiddelen of metalen en aangeboren aandoeningen.

In **Hoofdstuk 3** wordt het verband tussen maternale beroepsmatige blootstelling en schisis verder onderzocht in een patiënt-controle studie. In dit onderzoek werd specifiek gekeken naar blootstelling aan oplosmiddelen, gewasbeschermingsmiddelen, metalen, biologisch stof, mineraal stof en gassen/rook vroeg in de zwangerschap. Voor deze studie werden kinderen met schisis geselecteerd uit de Eurocat Noord-Nederland (Eurocat NNL) database. Eurocat NNL is een langlopend onderzoek waarbij alle kinderen geboren met

een aangeboren aandoening en waarvan de moeder woont in een van de drie noordelijke provincies in aanmerking komt voor registratie. Ouders ontvangen een vragenlijst om aanvullende informatie te geven over de zwangerschap, hun gezondheid, medicijn gebruik, levensstijl en werk. Ongeveer twee derde van de ouders stuurde de ingevulde vragenlijst retour. Er werden 124 kinderen met een schisis van het gehemelte en 263 kinderen met een schisis van de lip met of zonder schisis van het gehemelte geïncludeerd. Daarnaast werden twee controlegroepen geselecteerd uit Eurocat NNL: (1) kinderen geboren met erfelijke aandoeningen (n=1135), en (2) kinderen geboren met andere niet erfelijke aangeboren aandoeningen (n=4356). Vierenveertig procent van de moeders die een kind kregen met schisis was blootgesteld aan één van de stoffen die bestudeerd werden in deze studie, tegenover 41% in de eerste controlegroep en 38% in de tweede controlegroep. De resultaten toonden dat maternale beroepsmatige blootstelling aan gewasbeschermingsmiddelen en biologisch stof vroeg in de zwangerschap mogelijk verband houden met het krijgen van een kind met schisis.

Het urogenitale stelsel ontwikkelt zich onder invloed van hormonen. Blootstelling aan stoffen die de hormoonhuishouding uit balans brengen (zogenaamde hormoon verstorende stoffen) kunnen leiden tot een afwijkende ontwikkeling van het urogenitale stelsel. Daarom is in **Hoofdstuk 4** het verband tussen maternale beroepsmatige blootstelling aan hormoon verstorende stoffen vroeg in de zwangerschap en urogenitale aandoeningen onderzocht in een patiënt-controle studie. Kinderen geboren met een nier- en/of urinewegaandoening of een hypospadie werden geselecteerd uit de Eurocat NNL registratie. Nier- en urinewegaandoeningen werden geclassificeerd in vier groepen: (I) aandoeningen aan het nierparenchym, (II) aandoeningen aan het urine verzamelsysteem, (III), aandoeningen aan de embryonale migratie van de nieren of andere urinewegaandoeningen, (IV) combinaties van nier- en urinewegaandoeningen. Als controlegroep werden kinderen zonder aangeboren aandoening geselecteerd uit de Lifelines cohort studie. In totaal werden 537 kinderen met nier- en/of urinewegaandoeningen geïncludeerd, 371 kinderen met hypospadie en 5602 controles. Drieëntwintig procent van de moeders die een kind kreeg met een urogenitale aandoening was blootgesteld aan één of meerdere hormoon verstorende stoffen ten opzichte van 20% van de controle moeders. In deze studie werd een mogelijk verband gevonden tussen maternale beroepsmatige blootstelling aan specifieke hormoon verstorende stoffen (organische oplosmiddelen / alkylfenolische verbindingen en weekmakers / benzofenonen

/ parabenen / siloxanen) en aandoeningen aan het urine verzamelsysteem of combinaties van nier- en urinewegaandoeningen.

In **Hoofdstuk 5** is het verband tussen maternale beroepsmatige blootstelling aan biologisch en mineraal stof, oplosmiddelen, gewasbeschermingsmiddelen en metalen en aangeboren hartafwijkingen onderzocht. Kinderen geboren met een hartafwijking werden geselecteerd uit de Eurocat NNL registratie en geclassificeerd in zeven hoofdgroepen hartafwijkingen. Op deze manier werd rekening gehouden met de diversiteit aan hartafwijkingen die elk een eigen onderliggend embryonaal ontwikkelingsmechanisme hebben. De zeven groepen waren: conotruncale hartafwijkingen, atrioventriculaire septum defecten, abnormale pulmonale veneuze connecties, linkszijdige of rechtszijdige ventriculaire uitstroombaan obstructies, septum defecten en complexe hartafwijkingen. Als controlegroep werden kinderen zonder aangeboren aandoeningen geselecteerd uit de Lifelines cohort studie. Er werden 1174 kinderen met hartafwijkingen en 5602 controles geïncludeerd. In totaal was 38% van de moeders die een kind kregen met een hartafwijking beroepsmatig blootgesteld ten opzichte van 36% van de moeders uit de controle groep. Deze studie liet zien dat maternale beroepsmatige bootstelling aan biologisch stof, mineraal stof en metalen vroeg in de zwangerschap de kans op het krijgen van een kind met een linkszijdige of rechtzijdige ventriculaire uitstroombaan obstructie of een septum defect mogelijk vergroot.

In **Hoofdstuk 6** is een patiënt-controle onderzoek gedaan om het verband tussen maternale beroepsmatige blootstelling aan oplosmiddelen en gastroschisis (een aandoening van de buikwand) te onderzoeken. Deze studie is gedaan in de Verenigde Staten met data van de National Birth Defects Prevention Study. Dit is een groot onderzoek naar aangeboren aandoeningen dat is gedaan in tien staten tussen 1997 en 2011. Moeders werden uitgenodigd om mee te doen aan een telefonisch interview waarbij zij werden gevraagd naar hun gezondheid, medicijn gebruik, levensstijl en werk drie maanden voor de zwangerschap en tijdens de zwangerschap. Ongeveer twee derde van de moeders stemde in met dit telefonische interview. In totaal werden 879 kinderen met gastroschisis en 7817 kinderen zonder een aangeboren aandoening geïncludeerd. Het percentage moeders dat werd blootgesteld aan oplosmiddelen tijdens de periconceptionele periode (een maand voor conceptie tot drie maanden na de conceptie) was 7.3% voor gastroschisis patiënten en 7.4% voor controle moeders. Er werd geen verband gevonden tussen maternale beroepsmatige blootstelling aan oplosmiddelen en gastroschisis in het nageslacht en ook geen blootstelling-respons relatie.

In **Hoofdstuk 7** worden de belangrijkste resultaten en methodologische uitdagingen van dit proefschrift bediscussieerd. Ook worden suggesties voor toekomstig onderzoek en de perspectieven met betrekking tot beroepsmatige gezondheid in de periconceptionele periode besproken. Uit de resultaten van dit proefschrift blijkt dat werkgevers een zorgvuldige risico-inventarisatie en evaluatie van deze blootstellingen dienen uit te voeren op het werkterrein. Hiernaast is het van belang dat werkende vrouwen zich laten informeren over de blootstellingen waarmee zij eventueel in aanraking komen op het werk. Ook moeten ze zich houden aan de bestaande protocollen om mogelijk schadelijke blootstellingen zo veel mogelijk te verminderen om zo het risico op aangeboren aandoeningen te reduceren. Een arbeidshygiënist en een bedrijfsarts op de werkvloer is zeer aan te bevelen om informatie in te winnen en/of twijfels te bespreken.

Het achteraf karakteriseren van beroepsmatige blootstelling vroeg in de zwangerschap is een uitdaging. Voor toekomstig onderzoek is het belangrijk om bij het selecteren van de meest optimale methode voor het schatten van beroepsmatige blootstelling rekening te houden met de hoeveelheid en soort beroepskarakteristieken die worden verzameld. Tot slot moeten onderzoekers zich bewust zijn van de verschillende typen vertekening die het gebruik van verschillende controlegroepen met zich mee kan brengen.

DANKWOORD (ACKNOWLEDGMENT)

Mijn proefschrift is af! Doordat het schrijven van dit proefschrift parallel liep met mijn master mastergeneeskunde was het niet altijd makkelijk om alle ballen in de lucht te houden. Daarom zijn er een aantal mensen die ik graag wil bedanken voor hun hulp, vertrouwen en steun.

Ten eerste was er geen onderzoek geweest zonder bereidwilligheid van ouders. Ik wil alle ouders bedanken die de tijd hebben genomen om mee te doen aan het Eurocat onderzoek, zonder jullie moeite kan er geen onderzoek naar aangeboren aandoeningen gedaan worden. I also want to thank all the participants of the National Birth Defects Prevention Study for the time they invested to participate in this study. Ook wil ik alle kinderen en hun ouders die hebben deelgenomen aan Lifelines bedanken.

Zonder mijn (co-)promoteren had ik dit werk niet kunnen doen. Bedankt voor jullie vertrouwen dat ik mijn MD/PhD traject tot een succesvol einde zou brengen, ook al zag ik dat zelf op sommige momenten niet meer zitten. Met veel plezier denk ik terug aan onze lunch- en dinerbesprekingen. Onze leerzame discussies mij hebben ontwikkeld tot waar ik nu ben.
Prof. dr. Boezen, Marike, dank voor je optimisme en doortastendheid. Door jou heb ik veel geleerd over leidinggeven, methodologie en epidemiologie.
Prof. dr. Kromhout, Hans, mijn kennis over beroepsmatige blootstellingen was nihil, maar dankzij jou herhaaldelijke en zorgvuldige uitleg ben ik dit steeds beter gaan begrijpen. Ondanks dat je in Utrecht werkt was je bereid meerdere keren naar het hoge Noorden af te reizen en kon ik je altijd bellen voor hulp.
Dr. de Walle, Hermien, dankjewel dat je voor mij klaar stond. Dankzij jouw jarenlange ervaring met onderzoek naar aangeboren afwijkingen is dit proefschrift tot stand gekomen. Je hebt me veel geleerd over epidemiologie en de (on)mogelijkheden van het doen van onderzoek naar aangeboren aandoeningen. Ook op persoonlijk vlak kon ik alles bij je kwijt.
Dr. van Kammen-Bergman, Jorieke, door jou onuitputtelijke kennis over aangeboren aandoeningen heb ik veel geleerd over menig aandoening die in dit proefschrift beschreven wordt. De kwaliteit van mijn manuscripten zijn een stuk verbeterd door jouw nauwgezette blik! Bedankt voor alle leuke gesprekken die we hebben gehad als kamergenoten en dat je altijd voor me klaar stond als sparpartner.

Graag wil ik de leden van mijn leescommissie bedanken voor het beoordelen van mijn proefschrift, thank you for the effort of judging my thesis: prof. dr. J.P. Bonde, prof. dr. A. Hoek en prof. dr. I.L. van Langen.

Naast Hermien en Jorieke wil ik ook alle andere lieve Eurocat dames bedanken, zonder jullie zou er geen Eurocat zijn. Bedankt voor jullie vertrouwen en interesse in mijn proefschrift, als ik vastliep was er altijd iemand bij wie ik even kon uitblazen om weer nieuwe inspiratie te krijgen. Daarnaast waren de dagelijkse koffiepauzes iets om naar uit te kijken. Bedenk een onderwerp en het is wel aanbod gekomen: van mannen tot BN'ers, van Boer zoekt Vrouw tot Down Town Abbey, en van Limburg tot Fryslân; alles werd besproken als een echte vrouwenclub.
Lieve Frouke, Ester en Marion op het secretariaat, zonder jullie werk en inzet zouden er geen ouders meer meedoen aan het Eurocat onderzoek. Beste Priscilla, we hebben niet veel samengewerkt, maar dankzij jouw case finding in de perifere ziekenhuizen konden er nog meer ouders worden aangeschreven. Beste Renée, altijd boordevol goede ideeën, bijvoorbeeld onze kerstspeurtocht. Ga naar de Bloemsingel, plak stickers met pijlen op willekeurige voorwerpen en je hebt gegarandeerd een hilarische ochtend. Beste Marian, dank voor je expertise op epidemiologische gebied, hulp bij mooie syntaxen en je inspiratie om te blijven hardlopen. Tot slot wil ik Nicole bedanken, een betere kamergenoot kon ik me niet wensen, mede dankzij jouw humor wordt een man in dit vrouwen gezelschap niet gemist. We hadden altijd gespreken over van alles en nog wat, puzzelden eindeloos op syntaxen en hielpen elkaar met Excel. Samen met Hermien ben je een wandelend logboek en kon ik met alle vragen over de Eurocat database bij jullie terecht.

Beste dr. Reefhuis, Jennita, bedankt voor de fantastische tijd in Amerika. Na 1,5 jaar co-schappen moest ik wel weer op gang komen met onderzoek, maar onder jouw leiding kwam mijn enthousiasme helemaal terug. Ik werd met open armen ontvangen en heb ik een geweldige tijd gehad in Atlanta. Niet alleen vond ik de leerzame discussies over onderzoek waardevol, ook denk ik met warme herinneringen aan terug aan de wandelingen met Todd, Otto, Dessa en jullie vrienden door de prachtige natuur rondom Atlanta.

Dear dr. Almli, Lynn, it was such a pleasure to work with you in Atlanta. You were always there for me and I could ask you everything. We had such interesting conversations during

our lunches at all those Asian restaurants. I loved meeting your wonderful family. Thanks for your endless enthusiasm!

Dr. Carissa Rocheleau and dr. Tania Desrosiers, although I never met you in person, I really appreciated all your feedback on my manuscript. You both learned me a lot.

I also want to thank Katie Arnold for your contribution on our manuscript, hopefully you will find some time to visit the Frisian Horses in the Netherlands. I also want to thank all my other colleagues at the CDC, Atlanta, for their warm welcome and lunches at Buford Highway. Elena, thank you for your hospitality, and many thanks to Anna for discovering Atlanta with me. Tot slot wil ik alle Dutchies in Atlanta bedanken, de weekenden met jullie maakte mijn verblijf in Atlanta veel gezelliger: borrelen, Superbowl beleven, Wie is de Mol avonden en hardlopen door Nashville. Lieve Emma, hopelijk zien we elkaar snel weer!

Beste Jelmer Prins en Nynke Smidt, zonder jullie hulp en kennis was het nooit gelukt om mijn systematic review op deze manier uit te voeren. Beste Roel Vermeulen, bedankt voor je kritisch blik waardoor ik gedwongen werd mijn methodiek goed te evalueren. Beste Gideon Du Marchie Sarvaas, bedankt voor je hulp bij de classificatie van de aangeboren hartafwijkingen: door jou kwamen de cases in mijn dataset tot leven. Ook wil ik de andere medeauteurs, Martie van Tongeren en Nicole Corsten-Janssen, bedanken voor jullie goede feedback op mijn manuscripten.

Beste prof. dr. A. Hoek, Annemieke, jij stond aan de start van mijn onderzoeksloopbaan tijdens mijn bachelor en masteronderzoek bij de fertiliteitsgeneeskunde. Erg fijn dat je er nu weer bij bent in de leescommissie. Mede door jou, dr. Henk Groen en de inspirerende gesprekken met Lydia en José is mijn enthousiasme voor onderzoek ontstaan. Beste Henk, via jou leerde ik Eurocat kennen en kreeg ik de kans om mijn onderzoek carrière naar een hoger niveau te tillen.

Thank you Kate McIntyre for all your editorial assistance. Due to your suggestions, the English language in this thesis is perfect. Dankjewel Lisette voor het inplannen van alle afspraken, etentjes en altijd gezellig gesprekjes.

Ook wil ik de Junior Scientific Masterclass en het Ubbo Emmius fonds bedanken voor het (financieel) mede mogelijk maken van mijn MD/PhD traject en het toekennen van de Talent Grant voor mijn onderzoek in de Verenigde Staten.

Beste gynaecologen en andere collega's van het Medisch Centrum Leeuwarden, het werken bij jullie tijdens het schrijven van dit proefschrift was een aangename en leerzame afwisseling. Bedankt voor de klinische ervaring en de motivatie om mijn proefschrift zo snel mogelijk af te maken om terug de kliniek in te kunnen. Ook wil ik Jorien Woolderink en Marjolein Kleppe bedanken voor de tijd die jullie mij hebben gegeven om eerste dit proefschrift af te maken alvorens te starten als ANIOS Obstetrie en Gynaecologie in het Martini ziekenhuis. Bedankt mijn lieve collega arts-assistenten, de gynaecologen, verloskundigen en verpleegkundigen voor het warme welkom en de plezierige werksfeer en gezellige borrels / diners.

Lieve Trada's, bedankt voor een super fijne studententijd! Van kroegavonden, verkleedpartijen en allerhande diners, tot clubweekenden en vakanties; wat een belevenissen allemaal. Waar we ook ter wereld waren, we wisten elkaar altijd te vinden. Het is jammer dat we elkaar nu minder vaak zien nu het serieuze leven begonnen is. Toch voelt het direct weer vanouds vertrouwd als we bij elkaar komen. Lieve Carolina en Martine, ontzettend leuk dat jullie mij kwamen opzoeken tijdens mijn onderzoekstijd in Atlanta!

Ook Dido en Rienk wil ik bedanken voor de feestelijke studententijd. Ondanks het vele samen studeren, hebben we ook ontzettend veel lol gehad en te gekke avonden beleefd. Het voordeel als enige vrouw in dit gezelschap van drie mannen was dat ik altijd weer veilig naar huis werd gebracht. Inmiddels zijn we alle vier dokter en zien we elkaar een stuk minder dan vroeger. Bedankt voor de alle afleiding en hulp als ik het even niet meer zag zitten. En Rienk bedankt voor de vele keren dat je mijn fiets hebt gerepareerd.

Leave freondinnen Esther, Femke, Helena, Marit, Marjolein, Tine, Tjitske, Selma en Ymkje, wat bijzonder dat wij al zo lang vriendinnen zijn. Na een belevenisvolle middelbare schoolperiode bleven wij een hechte vriendinnengroep, wat we ook gingen doen, waar we ook woonden, iedereen volgde een eigen pad. Ook al hadden jullie soms geen idee wat dit pad voor mij precies inhield (ik zou toch gewoon dokter worden?). Onze jaarlijkse weekenden weg, gezellig feestjes, zingevende gesprekken en jullie oprechte interesse hebben me erdoorheen gesleept. En nu kunnen jullie eindelijk lezen wat ik heb gedaan. Mijn speciale dank gaat uit naar Tine voor je hulp en geduld tijdens het vormgeven van dit proefschrift en dat je tijdens mijn verdediging naast me komt staan.

Leave Gerk en Lia, ik kan me geen betere schoonouders wensen. Bedankt voor jullie oprechte interesse in mijn proefschrift en voor jullie gastvrijheid als wij weer een weekend kwamen uitrusten, varen, hardlopen, fietsen, tafels timmeren of taarten bakken.

Leave Marije, ondanks dat we zusjes zijn, zijn we erg verschillend. Toch zijn we op een vergelijkbaar pad beland en word jij dierenarts. Fijn dat jij Anton aan je zijde hebt. Bedankt dat jullie er zijn.

Leave Heit en Mem, toen ik vroeger heit zijn proefschrift in de boekenkast zag staan, leek mij een heel proefschrift schrijven maar niks. Dokter zijn daarentegen leek me fantastisch door alle spannende verhalen waar heit mee thuis kwam. Tijdens mijn studie kwam ik erachter dat ik onderzoek doen en dingen uitzoeken stiekem toch erg interessant vond en zo ben ik dan toch een promotietraject ingerold. Misschien had het er ook mee te maken dat jullie proefschrift uit 1988 er ook erg onaantrekkelijk (zwart) uitzag. Gelukkig heb ik mijn creatieve genen van mem geërfd en is dit proefschrift een stuk kleurrijker geworden. Bedankt voor alles, jullie onvoorwaardelijke steun en de vrijheid om mijn eigen keuzes te maken in het leven.

Leave Tjerk, vanaf het begin in de studiebanken waar wij al beste vrienden. Van samen studeren tot samen feestvieren, het was altijd een groot feest met jou. Behalve onze Friese wortels delen wij onze interesse voor wetenschap en samen zijn we aan een MD/PhD traject begonnen. Later, tijdens de vele koffiepauzes en etentjes bleken wij ook onze liefde voor elkaar te delen. Door jouw kritische blik haal jij het beste in mij naar boven en jouw humor laat je me altijd weer lachen. Het leven met jou is prachtig!

CURRICULUM VITAE

Nynke Spinder was born on February 21st, 1993 in Amsterdam, the Netherlands. She grew up in the Frisian village Dronryp with her parents and younger sister. After she graduated from secondary school at the CSG Comenius in Leeuwarden in 2011, she studied Medicine at the University of Groningen. During her bachelor she became interested in doing research and she conducted a research project at the department of Obstetrics and Gynaecology at the University Medical Center of Groningen (UMCG). After she completed her bachelor in Medicine, she performed her research internship at the same department under supervision of prof. dr. A. Hoek.

She continued her research at Eurocat Northern Netherlands (department of Genetics, UMCG) and the department of Epidemiology of the UMCG which resulted in a successful application of an MD/PhD trajectory in 2015. Nynke combined her research with clinical rotations at the UMCG and Medical Center Leeuwarden. She did her final medical internship at the department of Obstetrics and Gynaecology, Medical Spectrum Twente. After finishing her master in Medicine, she continued her research in January 2020 at the Centers for Disease Control and Prevention (CDC) in Atlanta, United States, under supervision of dr. Jennita Reefhuis and dr. Lynn Almli. In May 2020 she completed her PhD and started working as resident (ANIOS) Obstetrics and Gynaecology at the Martini Hospital Groningen. Nynke lives together with Tjerk Hidde Hylkema in Groningen.

RESEARCH INSTITUTE SHARE

This thesis is published within the **Research Institute SHARE** (Science in Healthy Ageing and healthcaRE) of the University Medical Center Groningen / University of Groningen. Further information regarding the institute and its research can be obtained from our internet site: http://www.share.umcg.nl/

More recent theses can be found in the list below.
(supervisors are between brackets)

2020

Lu C (Congchao)
Physical activity and health in Dutch and Chinese children
(prof E Corpeleijn, prof R Stolk)

Oosterwijk A
From range of motion to function; loss of joint flexibility after burns: when is it a problem?
(prof CP van der Schans, dr LJ Mouton, dr MK Nieuwenhuis)

Veen MM van
Facial palsy; treatment, quality of life, and assessment
(prof PMN Werker, prof PU Dijkstra)

Mooij R
Improving quality of maternal and perinatal care in rural Tanzania
(prof J Stekelenburg, dr J van Dillen)

Bunt S
Frailty among older adults; exploring the social dimension
(prof CP van der Schans; prof BJM Steverink; dr JSM Hobbelen)

Mousavi SH
A step forward in running-related injuries
(prof J Zwerver, prof RL Diercks, dr JM Hijmans)
09.09.2020 PHR

Lip SV
The effects of preeclampsia on the maternal cardiovascular system; gene expression and its (epigenetic) regulation in experimental preeclamptic cardiovascular tissues and cells
(prof T Plösch, dr MM Faas, prof SA Scherjon)

Renting N
Clinical workplace learning today; how competency frameworks inform clinical workplace learning (and how they do not)
(prof ROB Gans, prof ADC Jaarsma, prof JCC Borlefs)

Rietkerk W
Tailoring care for older adults; understanding older adults goals and preferences
(prof SU Zuidema, prof JPJ Slaets, prof DL Gerritsen)

Aalst RTA van
An economic assessment of high-dose influenza vaccine
(prof MJ Postma, prof JC Wilschut)

Kuiper D
Implementation of the self-management of well-being interventions; determinants and effects
(prof R Sanderman, prof SA Reijneveld, prof BJM Steverink, dr MM Goedendorp)

Zwertbroek EF
Hypertension in pregnancy; timing of delivery and early screening
(prof CM Bilardo, prof SA Scherjon, dr MTM Faassen, dr H Groen)

Boer P de
Cost-effectiveness of vaccination strategies to protect older adults: focus on herpes zoster and influenza
(prof MJ Postma, prof JC Wilschut)

Buijs-Spanjers KR
Improving delirium education: the role of experiential learning in a serious game
(prof SEJA de Rooij, prof ADC Jaarsma)

Karsten MDA
Women's lifestyle and sexual function; The effects of a preconception intervention in women with obesity

(prof A Hoek, prof TJ Roseboom, dr H Groen)

Koops JC
Understanding nonmarital childbearing. The role of socio-economic background and ethnicity in Europe and North-America.
(prof AC Liefbroer, prof AMH Gauthier)

Wasir R
Moving forward to achieve universal health coverage in Indonesia: progress and challenges
(prof E Buskens, prof MJ Postma, dr W Goetsch)

Kramer T
How to develop a Grand Slam winner…; physical and psychological skills in Dutch junior tennis players
(dr MT Elferink-Gemser, prof C Visscher, dr BCH Huijgen)

Raven D
Where's the need? The use of specialist mental health services in adolescence and young adulthood
(prof AJ Oldehinkel, prof RA Schoevers, dr F Jörg)

Stoter IK
Staying on track; the road to elite performance in 1500 m speed skating
(dr MT Elferink-Gemser, prof C Visscher, prof FJ Hettinga)

For earlier theses visit our website