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 \(^1\). 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 widespread 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%).
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). 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. 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. Several lifestyle factors can also increase the risk of congenital anomalies in offspring, including maternal and paternal smoking, alcohol consumption, and illicit drug use. In the general environment women can be exposed to air pollutants that can increase the risk of congenital anomalies. 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.

**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. 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.

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. 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. 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](image.png)

**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, 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. Only one study has reported an association between maternal occupational exposure to solvents and gastroschisis.

**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. 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. 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. The aim of Lifelines is to obtain insight into healthy ageing. Participants were invited...
Introduction

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. 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. 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). 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. 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


