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

<table>
<thead>
<tr>
<th>Occupational exposures</th>
<th>Congenital anomalies</th>
<th>Neural tube defects</th>
<th>Congenital heart defects</th>
<th>Orofacial clefts</th>
<th>Hypospadias</th>
<th>Urinary anomalies</th>
<th>Gastrochisis</th>
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</thead>
<tbody>
<tr>
<td>Organic dust</td>
<td>+</td>
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<tr>
<td>Mineral dust</td>
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<tr>
<td>Gases and fumes</td>
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<tr>
<td>Metals</td>
<td>Meta-analysis</td>
<td>NC</td>
<td>+</td>
<td>+</td>
<td>NC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solvents</td>
<td>Meta-analysis</td>
<td>+</td>
<td>NC</td>
<td>+</td>
<td>NC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pesticides</td>
<td>Meta-analysis</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>+</td>
<td>=</td>
<td>=</td>
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<tr>
<td>PAHs</td>
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<tr>
<td>Phthalates / Benzophenones / Parabens / Siloxanes</td>
<td>+</td>
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</table>

/=+=/ 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. 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.

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, 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, but the results of two other studies were not in line with our finding. 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. 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.

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. Second, circumstances at the workplace are often unpredictable and can vary within a job and between companies. 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.

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. 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. 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. The NBDPS abstracted clinical information from medical records, which was then reviewed by clinical geneticists using a systematic study-wide classification protocol. 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. 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. 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. The control participants of the NBDPS are representative of their base population. 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.39 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
Chapter 7

analysis. 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.
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